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Vaccinology

time to change the paradigm?

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Personal view paper

Vaccinology: Time to change paradigm?

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Abstract (226 words)

In the current vaccine paradigm, it is usually assumed that vaccines only protect against the target infection, that effective vaccines reduce mortality corresponding to the target infection's share of mortality, and that vaccine effects are similar for boys and girls. However, epidemiological vaccine research has generated observations, which contradict these assumptions and suggest that vaccines have important non-specific effects (NSEs).

We list eleven contradictory observations, including the observations that several live vaccines reduce mortality far more than can be explained by protection against the target infections, and that several non-live vaccines are associated with increased female mortality.

We suggest six principles, which may explain these observations: 1) live vaccines enhance resistance towards unrelated infections; 2) non-live vaccines enhance susceptibility towards unrelated infections for females; 3) the most recent vaccination has the strongest NSEs; 4) combinations of live and non-live vaccines given together have variable NSEs; 5) vaccinating with live vaccines in the presence of existing immunity enhances beneficial NSEs; and 6) vaccines may interact with other interventions affecting the immune system.

These principles may characterise a new paradigm: they may help resolve the contradictory observations, they raise new questions which could not have been seen from the existing paradigm, and they correctly predict new observations, which cannot be explained within the current paradigm. Pursuing these principles could lead to an optimised understanding and use of vaccines.

Introduction

Vaccines have enabled successful control of infections like smallpox, measles, polio, pertussis, and neonatal tetanus¹. When vaccines were introduced against smallpox and tuberculosis, some clinicians suggested that the vaccines protected against other diseases^{2,3}. However, this idea waned and left little imprint.

Instead, a vaccine paradigm has developed, according to which a vaccine is “a biological preparation that improves immunity to a particular disease”⁴. This paradigm guides how vaccines are evaluated and how vaccination policy decisions are made. For instance, vaccines are typically evaluated only for their protective effects against the target disease; their effect on general susceptibility to infections and on overall mortality is not measured. Furthermore, little attention is paid to sequence and combination of vaccines⁵, and vaccination policies are the same for boys and girls (Box 1).

A growing number of observations suggest that the current paradigm is not compatible with all data. In the terminology of Thomas Kuhn⁶, paradigm contradictions are accumulating. When that happens, we need new principles, which can resolve the contradictions.

In the following, we list eleven contradictions. Based on these contradictions, we propose that vaccines have also important *non-specific effects* (NSEs), and we discuss new principles, which can possibly help resolve the contradictions and point to new questions and ultimately a new vaccine paradigm.

Contradictions of the current vaccine paradigm

The contradictions (Box 1) deal with the effects of vaccines on overall health. They were initially detected by our group in Guinea-Bissau, but we have examined the replicability in other settings (Supplementary table 1). Few groups have studied the effect of vaccines on overall health; to ensure completeness, we searched Pubmed for studies of NSEs of vaccines.

The first contradiction was related to live standard MV. When routine measles vaccine (MV) was introduced in low-income countries in the 1970s-80s⁷, the >50% reductions in mortality were much larger than anticipated; at that time, ~10% of deaths were due to measles infection⁸⁻¹¹.

Subsequently, several randomised controlled trials (RCTs) showed that an additional early dose of MV was associated with major reductions in mortality^{12,13} (Table 1). These results contradicted that the only major effect of MV was to protect against measles infection (Contradiction I). Recently, it was suggested that the beneficial NSEs are due to MV preventing measles infection from inducing long-term loss of immune memory¹⁴. However, this hypothesis is contradicted by individual level data, which show that measles infection is associated with lower mortality after the acute phase¹⁵. Furthermore, MV is associated with mortality reductions in situations with little measles infection and thus no long-term memory loss to prevent¹³. In spite of no circulating measles infection, studies of measles-mumps-rubella (MMR) vaccine from high-income settings have also shown reduction of non-target infections, particularly respiratory infections¹⁶⁻¹⁹. A Dutch study found similar benefits for all vaccines, and suggested that NSEs represent “healthy vaccinee bias”¹⁸. However, MMR had a significantly better effect for respiratory infections than other vaccines²⁰.

Given the beneficial effects of standard MV, expectations were high when a live high-titre-measles-vaccine (HTMV) was introduced at 4-5 months of age in Africa in the 1980s. This vaccine clearly protected against measles infection²¹. However, long-term follow-up within RCTs in Guinea-Bissau, Senegal and The Gambia showed that girls, who received HTMV at 4-5 months, had two-fold higher mortality than girls in the control group, who received standard MV at 9-10 months of age²². In 1992, when replicated in Haiti²³, WHO withdrew HTMV²⁴. This contradicted that protective vaccines have beneficial effects and that vaccine effects are similar for boys and girls (Contradiction II).

Other live vaccines, however, also seemed to have beneficial NSEs. When BCG was introduced in Europe in the 1920s³ and in Guinea-Bissau in the 1980s²⁵, BCG was associated with major reductions in infant mortality even though TB mainly kills older children. Recently, the BCG-Denmark strain was tested in RCTs among low-weight children in Guinea-Bissau, who normally do not receive BCG at birth. BCG-Denmark-at-birth was associated with 38% (17-54%) lower neonatal mortality²⁶ (Table 1). These observations contradict that BCG only protects against TB (Contradiction III). However, the evidence is not uniformly consistent; two recent Indian trials

found no effect of the BCG-Russian strain on neonatal mortality²⁷ (Table 1), and a Danish trial found no effect of BCG-Denmark on infectious disease admissions²⁸.

Live oral polio vaccine (OPV) was also shown to have beneficial effects on overall survival, which cannot be explained by prevention of polio. In recent decades, there has been little wild polio infection. Nonetheless, the many national campaigns conducted to eradicate polio in West Africa have been associated with major reductions in child mortality²⁹⁻³². Furthermore, in an RCT, OPV-at-birth was associated with 32% (0-55%) lower infant mortality before the children received campaign-OPV³³ (Table 1). In Denmark, routine OPV was associated with fewer admissions for respiratory infections³⁴. RCTs comparing OPV versus inactivated polio vaccine (IPV) in Bangladesh and Finland found OPV to be associated with lower risk of diarrhoea³⁵ and otitis media³⁶. These observations contradict that OPV only protects against polio infection (Contradiction IV) and question the plan to replace OPV with IPV.

Yet another live vaccine, smallpox vaccine was associated with reductions in mortality. The decision to stop smallpox vaccination globally in 1980 was based on the assumption that smallpox vaccine only protected against smallpox infection. However, subsequent studies in Guinea-Bissau³⁷ and Denmark³⁸ of cohorts that experienced the phase-out of smallpox vaccine, showed lower long-term mortality after smallpox-vaccination (Contradiction V).

Recently, we have explored the determinants of beneficial NSEs of live vaccines. That led to the discovery of two further contradictions.

First, MV in the presence of maternal immunity improves the benefit of vaccination (Contradiction VI). It is well known, that MV given to infants with maternal measles antibody, may reduce the measles-specific antibody response; the age of MV was determined to shun maternal antibody^{7 39}⁴⁰. However, the beneficial impact of MV on survival turned out to be stronger when MV is given before age 12 months⁴¹. Furthermore, mortality between 4.5 months and 5 years was 78% (36-93%) lower if the child received early MV at 4.5 months of age in the presence rather than the absence of maternal measles antibody⁴².

Second, though the first dose of live vaccines usually protects against death from the target disease, we observed that revaccination with live vaccines are associated with marked mortality

reductions (Contradiction VII). For instance, campaigns with MV and OPV are conducted to reach unvaccinated children and a few non-responders, and are not assumed to substantially reduce all-cause mortality⁴³. However, studies from Guinea-Bissau^{30,43-45}, Ghana⁴⁶, Burkina Faso³¹, Bangladesh⁴⁴, Haiti⁴⁴, and Algeria⁴⁷ suggest that revaccination or campaigns with live vaccines (MV, BCG, OPV, and smallpox vaccine) reduce mortality substantially⁴⁴. In Denmark, a second dose of MMR was associated with lower risk of admissions for severe non-targeted infections⁴⁸.

In contrast, the non-live diphtheria-tetanus-pertussis (DTP) vaccine was associated with increased overall mortality for females (Contradiction VIII).⁴⁹⁻⁵¹ There are now 10 studies with prospective follow-up comparing DTP-vaccinated vs. DTP-unvaccinated children. In a combined analysis, DTP-vaccinated children had 2.07(1.60-2.67) times higher mortality than DTP-unvaccinated children^{49,50,52}. There are 17 studies of the female-male-mortality ratio among DTP-vaccinated children; in a combined analysis, females have 1.47(1.18-1.84) higher mortality than males^{49,50,53} (Table 2).

As for DTP, receipt of IPV⁵⁴, hepatitis B vaccine (HBV)⁵⁵, pentavalent (DTP+HBV+*H. influenzae* b) vaccine^{56,57} and H1N1 influenza vaccine³⁰ have been associated with higher female than male mortality (Contradiction IX, Table 2). Recently, a non-live malaria vaccine provided 18-36% protection against clinical malaria⁵⁸, but was associated with two-fold increased female mortality^{59,60}. Most studies finding excess female mortality after non-live vaccine were conducted in West Africa. There was no excess female mortality in the pre-vaccination era in West Africa⁶¹; vaccine coverage is usually similar for boys and girls⁶¹⁻⁶³, and excess female mortality is not seen in age groups dominated by live vaccines. Hence, excess female mortality is “unnatural”, and contradicts the assumption that vaccines benefit both sexes similarly.

In the current paradigm, sequence and combination of vaccines matter little; e.g. it makes little difference to pertussis or measles immunity whether DTP is given before MV, MV before DTP, or DTP and MV together. However, in all studies, mortality is increased if DTP is administered after MV compared with MV administered after DTP^{32,61,64,65}. Likewise, DTP given with MV is associated with higher mortality than having MV-only⁶⁴. Furthermore, children who received BCG+DTP, had lower mortality than children who got BCG-then-DTP as recommended^{64,66-68}. Also, co-administration of DTP and OPV is associated with lower mortality than DTP-only⁴⁹. In Denmark,

receiving DTaP-IPV-Hib with MMR vs. MMR-alone was associated with increased risk of admission for respiratory infections⁶⁹; in the US, receiving live vaccines together with non-live vaccines was associated with higher risk of admission for non-targeted infections than having a live vaccine only¹⁹. Thus, the sequence and combination of vaccines may matter greatly for child survival (Contradiction X).

The last contradiction relates to the interaction between vaccines and other health interventions. In the 1980s, studies investigated whether high-dose VAS could reduce child mortality. In a large RCT in northern Ghana, VAS versus placebo was associated with 19% (2-32%) reduction in child mortality⁷⁰. VAS became global policy and was piggy-backed on the vaccination program, but it was never examined whether VAS and vaccines interacted. In a reanalysis of the Ghanaian trial, VAS only benefitted children who had no vaccination card. Among children with a card, VAS vs placebo was associated with sex-differential effects, being less beneficial for females⁷¹. Several other studies support that the effect of VAS depends on vaccinations status in a sex-differential manner⁷²⁻⁷⁸. Hence, the assumption that vaccines only protect against the target disease was once again contradicted (Contradiction XI).

Resolving the contradictions: New principles

In situations with accumulating contradictions, the way forward is to formulate principles that can resolve the contradictions. This is new territory, and the approach therefore has to be iterative. The principles need to be tested on new data. Particularly their ability to provide accurate predictions should be assessed^{6,79}.

We have pursued a strategy of formulating deductions and testing them in all available data (Box 2, supplementary table 1). Sometimes the studies had limited power, but the important criterion has been consistency, i.e. patterns rather than p-values. If a principle did not predict outcomes, it was reformulated and tested again. If a principle lead to accurate predictions, it was preserved in the pursuit of a better paradigm.

The first principle is that live vaccines enhance resistance towards unrelated infections (Box 1). This principle explains the unexpected strong beneficial effects of MV, OPV, BCG and smallpox vaccines (Contradictions I+III-V). These benefits could partly be due to increased availability of

diagnostics and treatment for vaccinated vs. unvaccinated children, or due to social selection bias. However, the effect of smallpox vaccine was tested in natural experiments during the phase out of smallpox vaccine, limiting the bias, and MV, OPV and BCG were tested in RCTs. Principle 1 does not mean that live vaccines always have a beneficial effect; other interventions may modify the effect. For example, the beneficial effects of early MV in RCTs was not observed if the child previously received VAS¹³, subsequently received DTP⁶⁴ or participated in OPV campaigns⁸⁰. Apparently these interventions modified the effect of early MV, either by removing the benefits of early MV or by benefitting the control group relatively more. This may explain why RCTs found no benefit of early MV in situations with numerous OPV campaigns⁸¹. Recent trials of BCG-Russia found no effect on overall mortality; noteworthy, BCG strains are different⁸², and may also have different NSEs⁸³.

One important implication of live vaccines having beneficial NSEs is that stopping a vaccine after eradication could have negative effects, as may have happened for the smallpox vaccine^{37,38} and may soon happen for OPV⁸⁴ and MV⁸⁵.

Immunology has supported this principle by showing that live vaccines can induce innate immune training, producing stronger pro-inflammatory responses to unrelated antigens⁸⁶. In a recent proof-of-principle experiment, BCG vaccination of human volunteers modified the immune response to subsequent challenge with yellow fever vaccine⁸⁷ and malaria⁸⁸; this was brought about by epigenetic modifications of innate immune cells. Heterologous immunity may also play a role⁸⁹.

The second principle emerging is that non-live vaccines enhance susceptibility towards unrelated infections for females. This would explain the negative effects for females of the non-live vaccines examined so far: DTP^{49-53,61}, IPV⁵⁴, HBV⁵⁵, Pentavalent vaccine⁵⁶, H1N1 influenza³⁰, and RTS,S⁶⁰ (Contradictions VIII-IX)(Table 2). The principle also enabled us to predict the negative effects of RTS,S in females⁵⁹(Box 2). Though this principle is obviously controversial, no study without survival bias has contradicted this pattern⁵² (Supplementary table 1). The observations have been dismissed because they are from observational studies with high risk of bias and not from RCTs. However, due to healthy vaccinee bias, the deleterious estimates of DTP's effect are likely to be conservative⁵².

Immunology has supported this principle; non-live influenza vaccine and non-replicating modified *Vaccinia Ankara* were associated with reduced responses to unrelated pathogens, indicating some degree of tolerance^{90 91}. In a recent experiment, DTP induced immunotolerance to unrelated antigens 3 months post-vaccination which was partially restored by concurrent or subsequent BCG vaccination⁹². In the first RCT designed to study the heterologous immunological effects of DTP in both sexes, DTP-vaccinated females, but not males, exhibited downregulation of predominantly type 1 interferon genes and suppressed T-cell reactivity⁹³. Such immunological effects could help explain why non-live vaccines could lead to increased susceptibility to unrelated infections.

The third principle is that the most recent vaccination has the strongest NSEs. This principle explains why many studies have shown that non-live vaccines administered after live vaccines are associated with increased female mortality^{32,61,64}(Contradictions VIII-X) and live vaccines after non-live are associated with lower mortality (Contradictions I+III-V). It also explains why the live HTMV was associated with increased female mortality in several RCTs (Contradiction II)²². HTMV was given at 4-5 months whereas standard MV in the control group was given at 9-10 months of age. Few children had received three doses of DTP before 4-5 months of age, so most HTMV children received DTP after HTMV, whereas fewer control children received DTP after MV. The difference in sequence explained the difference in female mortality; increased female mortality was only seen if DTP was given after HTMV⁹⁴.

The fourth principle acknowledges that combinations of live and non-live vaccines given together have variable NSEs. It was found that MV (live last) is better than DTP+MV (combined)⁶⁴; BCG+DTP (combined) is better than BCG-then-DTP (non-live last)⁶⁴; and OPV (live last) is better than DTP+OPV (combined)⁹⁵. The first comparison, MV vs. DTP+MV, typically compared children following vs. not following recommendations, and thus the comparison could be biased in favour of MV. However, the two latter comparisons find lowest mortality in those who do not follow recommendations, so bias cannot explain the findings. The totality of data fits well with the pattern that combined live and non-live vaccines are worse than live vaccine only, but the combination is better than non-live vaccine only.

The fifth principle, that vaccinating with live vaccines in the presence of existing immunity enhances beneficial NSEs, may explain several contradictions. MV in the presence of maternal

antibody was associated with much better survival (Contradiction VI) and reviewing the literature, the beneficial NSEs of MV, BCG, and OPV were consistently stronger when the vaccines were administered early in life, when there would be more maternal antibody^{13,26,33,41}. The potential role of maternal priming was strengthened by the Danish BCG-Denmark trial²⁸. Among BCG-vaccinated mothers, randomisation of their child to BCG vaccination was associated with lower risk of admission for infectious diseases²⁸, GP visits⁹⁶, parental reported infections⁹⁶ and atopic dermatitis⁹⁷. Subsequent exploration of the hypothesis in Guinea-Bissau confirmed that mortality was lower for children having a BCG scar vs. children without a BCG scar if the mother also had a BCG-scar⁹⁸.

A recent immunological study from Uganda showed that maternal BCG scar was associated with upregulation of non-specific responses after the child had been BCG vaccinated⁹⁹. Though more studies are warranted, the available information suggests that priming from maternal immunity followed by child vaccination with the same pathogen strengthen resistance towards unrelated infections.

Revaccination with live vaccines is another way of vaccinating in the presence of pre-existing immunity, and is also associated with strong survival benefits (Contradiction VII). Thus, this principle may explain the enormous effects that repeated campaigns with OPV and MV have had on mortality in the last 20 years^{29-32,43,45}.

The sixth and last principle is that vaccines may interact with other interventions affecting the immune system. VAS is an immunomodulator^{77,100-102}. When the VAS study was conducted in Ghana in 1989-1991, most children received DTP with MV or DTP after MV^{32,71}. The negative effect of VAS for girls with a vaccination card was predominantly seen in children who got DTP; thus, VAS may have amplified the negative effect of DTP in girls⁷¹. Hence, principles 2, 3, 4 and 6 all contribute to resolving Contradiction XI. The negative interaction between VAS and DTP was also found in recent studies of neonatal VAS; once the children got DTP, neonatal VAS became associated with increased female mortality^{77,103}.

Discussion and conclusions

The claims in this paper are bold and their potential implications for child health are huge; for example, if BCG was given earlier, if a higher coverage of MV was obtained, if DTP-containing

vaccines were not given with or after MV, and if the BCG strain with the best NSEs was used consistently, child mortality level could be considerably lower¹⁰⁴.

The HTMV observations were based on RCTs and the mortality implications were major, so there should be no doubt that NSEs exist and can be very important. Unfortunately, there have been few attempts to pursue NSEs. It has been claimed that only RCTs can ascertain whether there are important NSEs¹⁰⁵. However, an RCT withholding (or delaying) a vaccine already recommended would not be approved by an ethics committee. Hence, insisting on RCTs can uphold further testing. Fortunately, there are other ways to establish causality^{15,106}; triangulation of evidence from RCTs, natural experiments and other observational studies, using different designs with different underlying confounding structures, can facilitate causal inference¹⁵.

The six principles are only a beginning. There are still inconsistent observations to be pursued (Box 3). This may open new avenues of prevention and treatment. Immunology has supported principles 1 and 2; mechanistic explanations for the other principles are being examined^{86,90,93,100,107}. As more new questions are being asked, new principles will likely be found and new mechanistic explanations will be needed. The results will have direct implications for vaccine developers. In 2015, vaccine producers and researchers discussed the potential off-label use of existing vaccines, and implications for new vaccines being developed¹⁰⁸. Two new live vaccines under development, an intra-nasal pertussis vaccine¹⁰⁹ and a new oral *S. Typhi* vaccine¹¹⁰, have been tested for their NSEs; both appeared to have beneficial NSEs^{109,110}. The finding that vaccines affect the innate immune system have also opened a new avenue in vaccinology: to develop *Trained Immunity-based Vaccines*, defined as vaccine formulations that induce training in innate immune cells¹¹¹.

A new paradigm should resolve contradictions in the current paradigm. Furthermore, it should lead to better predictions and to new questions, which would not otherwise have been asked. The six principles have already led to predictions that could be confirmed. For example, principle 1 raised the question whether the numerous OPV campaigns reduced the general mortality rate (which they did)²⁷⁻³⁰ and principle 2 raised the question whether RTS,S malaria vaccine was associated with higher female mortality (which it was)⁶⁰ (Box 2).

Based on evidence, we have put forward another six testable predictions (Box 2). E.g., we predict that the planned replacement of live OPV with non-live IPV and stopping OPV campaigns will lead to increased child mortality. If any of these predictions are confirmed, it would collide with the assumptions of the current paradigm, and support the proposed principles.

It has been estimated that taking NSEs into account in the vaccination program could reduce global mortality by 1.1 mill deaths per year¹¹². This could also reduce health care costs in richer nations. Effective changes would help to redefine the debate on vaccine hesitancy and likely increase confidence and improve vaccine coverage.

With the numerous contradictions in vaccinology it seems high time to change the paradigm and construct a better guide to the future. We posit that the six principles stated here could be the starting point for further studies with the promising potential of using existing vaccines more effectively.

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Table 1. Randomised controlled trials (RCTs) of live vaccines with information on mortality reductions due to specific disease protection and mortality reductions due to non-specific effects of vaccines

Vaccine	Country, design, follow-up period	Reduction in mortality from the targeted infection	Reduction in mortality from non-targeted infections
BCG vaccine			
BCG-Denmark ²⁶	Guinea-Bissau, Combined analysis of three RCTs of BCG-Denmark+OPV vs OPV at birth, 0-4 weeks	0%	38% (95% CI: 17-54%)
BCG-Russia ²⁷	India, Combined analysis of two RCTs of BCG-Russia with and without OPV vs no BCG or OPV, 0-4 weeks	0%	2% (95% CI: -11-15%)
BCG-Denmark ¹¹³	Guinea-Bissau, A second dose of BCG at 19 months given after a booster dose of DTP vs no second BCG (secondary endpoint), 19 months to 5 years	0%	64% (95% CI: 1-87%)
Oral Polio Vaccine (OPV)			
OPV-at-birth ³³	Guinea-Bissau, OPV+BCG vs BCG at birth, 0-11 months	0%	32% (95% CI: 0-57%)
Measles vaccine (MV)			
Measles vaccine ⁹⁴	Sudan, Senegal, The Gambia, Guinea-Bissau, Medium or high-titre MV vs control vaccine, 4-8 months	0%	60% (95% CI: -8-85%)
Measles vaccine ¹²	Guinea-Bissau, MV at 6+9 months vs IPV at 6+MV at 9 months, 6-8 months	0%	70% (95% CI: 13-92%)
Measles vaccine ¹³	Guinea-Bissau, MV at 4.5+9 months vs no vaccine at 4.5+MV at 9 month, 4.5 months-3 years	4%	22% (95% CI: -5-41%)
Measles vaccine ⁸¹	Burkina Faso, Guinea-Bissau, MV at 4+9 months vs no vaccine at 4+MV at 9 month, 4 months-3 years	0%	-5% (95% CI: -32-33%)

Abbreviations: BCG, bacille Calmette Guérin; RCT, randomised controlled trials; OPV, oral polio vaccine; MV, measles vaccine; IPV, inactivated polio vaccine.

Table 2. Relative risks (RR) of overall and female mortality associated with non-live vaccines

Vaccine	Studies	Females and males combined: RR of mortality for vaccinated vs. unvaccinated	Females only: RR of mortality for vaccinated vs. unvaccinated	RR of mortality for females vs. males among vaccinated children
Diphtheria-tetanus-pertussis vaccine	17 observational studies	2.07 (1.60-2.67) ^{49,50,52}	2.54 (1.68-3.86) ⁵³	1.47 (1.18-1.84) ^{49,50,53}
RTS,S malaria vaccine	Two RCTs	1.24 (0.97-1.58) ^{58,59}	RCT-1: 1.81 (1.04-3.14) ⁶⁰ RCT-2: 2.00 (1.18-3.39) ⁶⁰	1.33 (1.02-1.74) ⁶⁰
Inactivated polio vaccine	Three RCTs	NA	NA	1.52 (1.02-2.28) ⁵⁴
Hepatitis B vaccine	Natural experiment	1.81 (1.19-2.75) ⁵⁵	2.27 (1.31-3.94) ⁵⁵	2.20 (1.07-4.54) ⁵⁵
Pentavalent vaccine	Observational study	NA	NA	1.73 (1.11-2.70) ⁵⁶
H1N1 influenza vaccine	Natural experiment: Campaign	1.86 (1.02-3.42) ³⁰	2.32 (1.19-4.52) ³⁰	2.68 (0.44-16.4) ³⁰

NA: No data available on unvaccinated. Abbreviations: RCT, randomised controlled trials. RRs were calculated using the “meta” command in Stata.

Box 1. Overview of the current assumptions about vaccines, the contradictions of these assumptions, and the emerging principles which may help resolve the contradictions

Current assumptions about vaccines	Eleven contradictions of the current paradigm	Six emerging principles
<p>Vaccines induce protective immunity against the target disease.</p> <p>Vaccines have no effect on the risk of other infections.</p> <p>Vaccines have similar effects in males and females</p> <p>Once a vaccine infection has been eradicated, the vaccine can be removed without health consequences</p>	<p>The live vaccines standard MV, BCG vaccine, OPV and smallpox vaccine have beneficial effects on overall survival, which cannot be explained by prevention of measles infection (contradiction I, III, IV, V)</p> <p>The non-live vaccines DTP vaccine, pentavalent vaccine, hepatitis B vaccine, IPV, H1N1 vaccine, and RTS,S malaria vaccine are associated with increased overall mortality for females (contradiction VIII-IX)</p>	<p>Principle 1: Live vaccines enhance resistance towards unrelated infections</p> <p>Principle 2. Non-live vaccines enhance susceptibility towards unrelated infections for females</p>
<p>The sequence and combination of vaccines matter little if no interference with specific protection</p>	<p>The live high-titre measles vaccine was associated with increased female mortality despite protecting against measles infection (contradiction II); it turned out to be due to non-live vaccines given afterwards</p> <p>The sequence and combination of vaccines may change the overall mortality effect (contradiction X)</p>	<p>Principle 3. The most recent vaccination has the strongest NSEs</p> <p>Principle 4. Combinations of live and non-live vaccines given together have variable NSEs</p>
<p>Maternal antibody provide temporary protection to the child, but reduce the antibody response to vaccination</p>	<p>Measles vaccination in the presence of maternal immunity does not reduce the survival benefit (contradiction VI)</p>	<p>Principle 5: Vaccinating with live vaccines in the presence of existing immunity enhances beneficial NSEs</p>

Little additional survival benefits expected from revaccination with live vaccines	Revaccination with live vaccines are associated with marked mortality reductions (contradiction VII)	
Vaccines do not affect the impact of other immunomodulatory health interventions	The effect of high-dose vitamin A supplementation depends on vaccination status (contradiction XI)	Principle 6. Vaccines may interact with other interventions affecting the immune system

Abbreviations: MV, measles vaccine; BCG, bacille Calmette Guérin; OPV, oral polio vaccine; DTP, diphtheria-tetanus-pertussis vaccine; IPV, inactivated polio vaccine.

Box 2. Deductions derived from the emerging principles of non-specific effects (NSEs) of vaccines which have been or can be tested

DEDUCTIONS WHERE SOME EVIDENCE IS AVAILABLE		
Initial observation	Deduction made	Result of testing
Live MV had beneficial NSEs in low-income countries	BCG, OPV and smallpox vaccine could also have beneficial NSEs	Supported by both observational studies and RCTs of BCG and OPV ^{26,33,35-38}
Live MV had beneficial NSEs in low-income countries	MMR could be associated with reductions in hospital admissions in high-income countries	MMR has been reported to be associated with reduction in respiratory infections in Denmark ^{16,114} , US ¹⁹ , Italy ¹⁷ , and The Netherlands ^{18,20}
Live vaccines have beneficial NSEs	Eradication of the target infection and stopping the live vaccine could have negative effects	In several studies from Guinea-Bissau and Denmark, smallpox vaccinated individuals had lower morbidity and mortality than unvaccinated individuals ^{37,38}
Live vaccines have beneficial NSEs	Campaigns with live vaccines to eradicate a disease might have strong beneficial NSEs even though the target infection is nearly eliminated	Strong beneficial effects have been shown for OPV and MV campaigns ^{29-32,43,45} whereas other campaigns with VAS or H1N1 influenza do not have such beneficial effects ³⁰
Non-live DTP has negative NSEs for females	Other non-live vaccines might also have negative effects for females	So far seen for IPV ⁵⁴ , HBV ⁵⁵ , Pentavalent ⁵⁶ and H1N1 influenza vaccine ³⁰
RTS,S provided moderate protection against clinical malaria but overall mortality tended to be higher in the RTS,S group ⁵⁸	We predicted that RTS,S might be associated with higher female mortality ⁵⁹	RTS,S turned out to be associated with 2-fold higher mortality for females in both age groups in which the vaccine had been tested ⁶⁰
DEDUCTIONS THAT CAN BE TESTED		
Current state-of-the-art	Deduction made	Ways to test
When OPV is stopped in 2024 and replaced by IPV, it will have no measurable impact on overall mortality	Replacing OPV with IPV will increase overall mortality (Principles 1 and 2)	RCT comparing OPV vaccination schedule with IPV vaccination schedule on overall mortality and morbidity
When OPV campaigns are stopped it will have no measurable impact on overall mortality	Stopping OPV campaigns will increase under-five mortality or at least reduce the rate of decline in mortality (Principle 1)	RCT testing the effect of conducting an OPV campaign on overall mortality and morbidity

Providing RTS,S in a trial of 720,000 children in Africa will reduce their overall mortality by reducing malaria mortality	RTS,S-vaccinated females will have higher mortality than RTS,S unvaccinated females and higher mortality than RTS,S-vaccinated males (Principle 2)	Collect information on overall mortality data in ongoing RTS,S trial by means of individual levels follow-up rather than by means of community informants and pharmacovigilance.
SAGE is recommending the introduction of a 2 nd year of life platform for additional (mainly non-live) vaccinations to reduce overall mortality ¹¹⁵	Introduction of more non-live vaccines in 2 nd year of life will increase female versus male mortality (Principles 2 and 3)	The introduction of new vaccines should be done in a randomised fashion, allowing unbiased assessment of the overall mortality and morbidity effect. In addition, observational studies of female-male overall mortality and morbidity ratios according to most recent vaccine
Giving a second dose of BCG with the third priming dose of DTP-containing vaccine at 14 weeks would have little or no effect on all-cause mortality	A second dose of BCG at 14 weeks would substantially reduce mortality between 14 weeks and 9 months of age (when measles vaccine is given) (Principle 4)	RCT testing the effect of providing a second BCG vaccine together with the third dose of DTP-containing vaccine at 14 weeks of age on overall mortality and morbidity
Whether the mother has a BCG-scar or not is unlikely to affect the mortality of the child	BCG vaccinated children will have substantially lower mortality if their mother have a BCG-scar than if she does not (Principle 5).	RCT testing the effect of providing a BCG vaccine to women in fertile age on overall mortality and morbidity in their child after BCG vaccination.

Abbreviations: NSE, non-specific effects of vaccines; BCG, bacille Calmette Guérin; RCT, randomised controlled trials; OPV, oral polio vaccine; MV, measles vaccine; MMR, measles-mumps-rubella vaccine; VAS, vitamin A supplementation; DTP, diphtheria-tetanus-pertussis vaccine; IPV, inactivated polio vaccine; HBV, hepatitis B vaccine; Pentavalent, DTP + Hepatitis B + *H. influenzae* b; SAGE, Strategic Advisory Group of Experts on Immunization (under the World Health Organization).

Box 3. Inconsistencies in the new principles which should be pursued

Inconsistency in principle	Potential explanation to pursue
Different BCG vaccines have had variable effect on both TB specific outcomes and non-specific outcomes ^{83,116,117}	There is a need to assess which BCG strains are most efficacious for both specific and non-specific outcomes ^{83,116}
Though we have emphasised that the most recent vaccine has the strongest NSEs, some vaccines appear to have long-lasting NSEs; e.g. children having a BCG-scar or adults having smallpox scars appear to have long-term survival benefits ^{37,38,118}	Further mechanistic research is needed into how vaccines may induce long-term imprinting effects. In relation to BCG this may be due to effects of BCG on bone marrow progenitor cells
The sex-differential NSEs found in low-income countries do not seem to apply when the NSEs of vaccines on morbidity have been studied in high-income settings; e.g. the stronger beneficial NSEs of MV for girls found in low-income countries was not found for MMR in Denmark ¹⁶	Boys have a higher incidence of admissions in Denmark, and they may have more to gain from MMR ¹⁶
In contradiction of principle 2, it has been hypothesised that non-live rabies vaccine has beneficial NSEs ^{119,120} . The main basis for this hypothesis is that the control group in one of the RTS,S trials received rabies vaccine and did better than the RTS,S group	This hypothesis is being pursued

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