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Review

How to evaluate potential non-specific effects of vaccines: The quest for randomized trials or time for triangulation?

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Abstract

Introduction: Emerging evidence suggests that vaccines, in addition to their disease-specific effects, have important non-specific effects (NSEs), which contribute to their overall effect on mortality and morbidity. Immunological studies have shown that NSEs are biologically plausible. Many advocate that randomized controlled trials (RCTs) with overall mortality or morbidity as the outcome are the only way forward to confirm or refute NSEs.

Areas covered: We discuss the limitations of using RCTs only as a tool to evaluate NSEs of vaccines. Such RCTs can be ethically problematic, they are time consuming and expensive. Furthermore, they only assess the NSEs in a given context, but it is inherent in the concept of NSEs that the NSEs of a given vaccine are modified by other immunomodulatory conditions. As an alternative, we propose that triangulation of RCTs and observational studies, merging multiple lines of evidence with different underlying bias structures, can build a strong argument for causality. We examine two examples related to measles vaccine and oral polio vaccine.

Expert commentary: Using RCTs alone to evaluate NSEs of vaccines severely limits the possibilities for studying NSEs. Results from both RCTs and non-RCT studies should be triangulated to strengthen causal interpretation.

Keywords: vaccines; non-specific effects; child mortality; child morbidity; randomized controlled trials; observational studies; evaluation; triangulation; vaccine policy.
1. Introduction

There is increasing evidence that vaccines have important non-specific effects (NSEs), i.e. that they affect susceptibility to unrelated infections, with important effects on overall mortality and morbidity[1].

WHO recently reviewed the evidence for NSEs on mortality of BCG, measles vaccine (MV) and diphtheria-tetanus-pertussis (DTP) vaccine[2, 3], three of the key vaccines in the child immunization program (Figure 1). Because NSEs are most pronounced as long as a vaccine is the most recently received vaccine, the review aimed to capture effects before subsequent vaccines were given. The review found that the live BCG and MV were associated with marked reductions in all-cause mortality that were not explained by the specific effects of the vaccines[2, 3]. In contrast, the non-live DTP vaccine was associated with a tendency for increased mortality[2, 3]. The review further noted that the sequence or combination of live and non-live vaccines mattered. Receiving BCG out-of-sequence, i.e. BCG with or after DTP vs. DTP after BCG (currently recommended), was associated with lower mortality. Receiving DTP out-of-sequence, i.e. DTP with or after MV vs. MV after DTP (currently recommended), was associated with higher mortality[2, 3].

Immunological studies have added plausibility to the existence of NSEs, by showing that vaccines can train the innate immune system to increased resistance towards unrelated pathogens[4-6]. In a recent proof-of-principle experiment in humans, BCG given 28 days prior to a “challenge” yellow fever vaccination was associated with reduced yellow fever viraemia[7].

WHO’s Strategic Advisory Group of Experts on Immunizations (SAGE) concluded that as all observation studies were considered “at high risk of bias”, there was “insufficient evidence to
support changes to current global policy”[8]. SAGE recommended further research into the NSEs of vaccines[8]. SAGE discouraged observational studies, emphasizing that only data from randomized clinical trials (RCTs) had sufficient quality to change policy[8]. SAGE subsequently charged the Immunization and Vaccines related Implementation Research Advisory Committee (IVIR-AC) with prioritizing and developing future RCTs of NSEs[8].

Other researchers have expressed similar opinions, that RCTs are the ultimate epistemological standard for good data, and for settling disputes regarding the existence and importance of NSEs of vaccines[9]. This follows a general trend; as noted recently, epidemiology and the clinical sciences often focus on producing statistically significant, definitive studies centered around an endpoint that supports a hypothesis, rather than using multiple sources of information[10].

In the present review, we aim to discuss the validity of the viewpoint that RCTs alone will resolve the issue regarding NSEs of vaccines, and we will bring examples of other lines of reasoning, using triangulation as a strong tool to build causal inference and advance our understanding of vaccines.

2. Challenges with RCTs

While there is no doubt that RCTs are important, the medical literature hierarchy of study designs placing RCTs as the “gold standard”, has been criticized[11]. Randomized controlled trials can be subject to multiple errors[12, 13], and important scientific discoveries such as the evolution of species, planetary orbits, and the effects of cigarette smoking on human health comes from non-randomized studies[14]. Furthermore, RTCs are a very difficult “gold standard”. An exclusive focus on RCTs may ultimately be counterproductive for assessing NSEs and for using potential NSEs to improve human health.
2.1. **Challenges related to ethical issues**

- Many NSEs relate to routine vaccines, recommended by WHO, and it will often be considered unethical to test such vaccines in RCTs, since it would imply delaying or depriving some children of recommended vaccines. For example, to study the NSEs of BCG vaccine in children who would normally get BCG at birth would imply delaying BCG-vaccine in the intervention group, and that could put them at risk.
- Some NSEs relate to potential deleterious effects of vaccines. The ethics of informed consent and of benefits to participants, an integral part of RCTs, makes it difficult to test potential deleterious effects. RCTs of DTP, RTS,S malaria vaccine and other non-live vaccines associated with higher female mortality[15, 16] would face that problem.
- For ethical reasons, RCTs are often required to provide extensive medical benefits to trial participants. This means that the mortality profile is changed and the result may not be representative for children with limited access to health care, for whom the vaccine is assumed to have the largest effect.

2.2. **Challenges related to measuring a context independent effect**

- In the real world, RCTs of health interventions, which affect the immune system, do not provide definitive answers regarding a “true” effect, which is always valid and context independent. There are numerous possible effect modifiers, which may change the estimated effect. All results of such RCTs are therefore contextual, affected by known and unknown effect modifiers, and not necessarily applicable to all situations. For example, the RCTs of high-titre measles vaccine (HTMV) in the late 1980s concluded that this vaccine, which was protective against measles infection, was associated with higher female mortality. WHO withdrew HTMV in 1992. However, a reanalysis subsequently showed that the excess female
mortality was more likely due to inactivated vaccines being administered after HTMV. HTMV was administered at 4-5 months of age and most children got DTP and/or inactivated polio vaccine (IPV) after HTMV; these vaccines have been associated with higher female mortality and HTMV therefore became associated with excess female mortality[17].

2.3. **Challenges related to cost**

- RCTs large enough to measure the impact of a given vaccine on mortality until the next vaccine is provided are very costly. Likely, only large pharmaceutical companies will be able to fund such RCTs. This monopolized situation and the potential inherent conflict of interests will not be productive for detecting and using the NSEs. Alternatively, the cost can tempt researchers to emphasize longer follow-up periods where other routine vaccines are given, but the studies then do no longer provide an estimate of the NSEs of the vaccine in question, but an estimate of a mixed exposure of different vaccines.

2.4. **Challenges related to time**

- RCTs take time. The preparation phase, designing the study, getting ethical approval and funding, recruiting and training staff may well take several years. Subsequently follows years of enrolment and follow-up, data cleaning and analysis and publication. It could happen that during this period it is discovered that the study was designed inappropriately, or the conditions for the trial change, e.g. new interventions or treatments are introduced. So using RCTs, it is only possible to evaluate and modify and adapt health interventions with 5-10 years’ notice.
3. Alternatives to RCTs – two examples of building evidence for NSEs by triangulation

In view of the challenges related to RCTs, we need other ways of assessing whether vaccines and their combination or sequence are likely to have important NSEs. Triangulation is the practice of integrating results from several different approaches to obtain more reliable answers to research questions. Each approach has its own assumptions, strengths and weaknesses. If the results of different approaches all point to the same conclusion, this strengthens confidence in the finding. This is particularly the case when the key sources of bias of some of the approaches would predict that findings would point in opposite directions if they were due to such biases [10, 18].

A few examples may illustrate how evidence for NSEs can be built by triangulating evidence from RCTs and different observational study designs.

3.1. Example: Measles vaccine

The WHO review of NSEs of measles vaccine (MV) included four RCTs and 18 observational studies of MV[2]. The observational studies suggested that MV compared with no MV was associated with a relative risk (RR) for overall mortality of 0.51 (0.42-0.63), but all studies were considered at high risk of bias[2]. The four RCTs included in the WHO-review[19-22] gave a relative risk (RR) of 0.74 (0.51-1.07)[2]. SAGE concluded that “The available data suggest that the current WHO recommended schedule for current standard titre measles-containing vaccine has a beneficial effect on all-cause mortality in children”. However, it was also concluded that “the evidence does not support a change in policy for measles vaccine”, and further RCTs were requested[8]. This would not make sense, if the existing results were taken as proof of important NSEs of MV, in which case efforts should be made to provide MV earlier than the currently recommended age of 9 months, to further reduce child mortality[23]. In support of such a decision, it is now clear that
children born of measles vaccinated mothers often have no measurable maternal measles antibody left by 4-5 months of age, and can be successfully vaccinated[24-26]. Hence, there still seem to be uncertainty about the importance of NSEs of MV[9].

By merely focusing on the four RCTs, which yielded a non-significant meta-estimate, and by regarding observational studies comparing MV-vaccinated and MV-unvaccinated children of limited value, important information may have been overlooked:

First, the estimated MV effect in the RCTs may have been modified by DTP. Many studies have shown that DTP administered with or after MV will remove the beneficial NSEs of MV on mortality. The WHO-review reported that comparing DTP with MV vs. MV after DTP, a combined analysis of the five available studies yielded an RR of 2.29 (1.55-3.37); for DTP after MV compared with MV after DTP, based on three available studies the RR was 2.66 (1.04-6.81)[2]. In two of the four RCTs[20, 21] included in the SAGE review, the children were likely to have received DTP co-administered or with MV or after MV; the RR in these two trials of MV was 0.95 (0.55-1.63). In the other two RCTs, in which the children had not received DTP after MV because it was part of the design to have received DTP3 before enrolment[22] or because the study was conducted in the 1960s before DTP was introduced[19], the estimated RR was 0.60 (0.36-0.99). Noteworthy, the two community trials, conducted before DTP became common, which compared vaccinated and unvaccinated communities in Congo (RR=0.29 (0.09-0.98))[27] and Bangladesh (RR=0.51 (0.42-0.62))[28] also supported beneficial NSEs of MV on overall mortality.

Second, a beneficial effect of MV is also supported in the meta-analysis of five individually randomized RCTs of high-titre MV (HTMV) or medium-titre MV (MTMV) from Guinea-Bissau (N=2), Senegal, The Gambia and Sudan[15]. In these RCTs, children were randomized to early
HTMV/MTMV or comparator vaccine around 4-5 months of age. The measles-vaccinated children compared with not-yet measles-vaccinated controls had a RR of 0.20 (0.06-0.65) until they received subsequent doses of DTP[15].

Third, in the five observational studies from Guinea-Bissau[29], Senegal[30, 31], Congo[27] and India[32], documenting the general mortality rate before and after the introduction of MV, the reduction in mortality rate was at least 50% after the introduction of MV. The consistency across all the different sites supports that the explanation should be sought in the introduction of the MV - and not other contextual changes happening at the same time.

Fourth, even though the first dose of MV is usually protective against measles infection, boosting with a second dose of MV reduces mortality substantially in observational studies, RCTs and natural experiments with MV-campaigns[33].

Fifth, even though MV is equally protective against measles infection in the two sexes, MV has a stronger beneficial effect on overall survival for girls than boys[2]. Since DTP in the same societies is associated with the opposite effect, higher female than male mortality[34], the sex-differential effect of MV is not a cultural artefact from differential access to care.

Sixth, an RCT from Guinea-Bissau found individual randomization to early MV to be associated with a reduction in hospital admissions for respiratory infections (RR=0.37 (0.16-0.89))[35]. Subsequent register-based studies from high-income countries have found measles-mumps-rubella (MMR) vaccine to be associated with marked reductions for hospital admissions[36-40]. This was particularly seen for respiratory infections, so the relative benefit of MMR for respiratory infections vs. other infections was higher in all studies with available data (Table 1). Even though some of the reduction in admissions in these studies might be due partly to healthy vaccinee bias,
it does support a biological interpretation that all four high-income countries studies found stronger beneficial NSEs for respiratory infections than for other disease categories.

Thus, in addition to the information included in the WHO review, there is substantial additional support for MV having beneficial NSEs. Triangulation of the results of the four RCTs, the 18 observational studies, and the six observations above would suggest that MV and MMR have beneficial NSEs affecting respiratory infections and overall mortality substantially.

A Science paper by Mina et al.[41] modelled time-trend correlations between aggregated measures of incidence of measles infection and deaths in certain age groups in the US, UK and Denmark and suggested a possible explanation for the beneficial NSEs of MV: the models were compatible with measles infection inducing a long-term loss of immune memory and consequent increased mortality. Based on this it was hypothesized that the observed mortality reductions after MV could be explained by MV preventing long-term loss of immune memory due to measles infection (“indirect non-specific benefits”)[42]. The authors emphasize that this explanation does not exclude direct beneficial effects of MV on the immune system [42]. However, though the two hypotheses are not mutually exclusive, there are several problems with the “indirect non-specific benefits” hypothesis. First, for the hypothesis to be true, individuals who survived the acute phase of measles infection should subsequently have higher mortality. In fact, in the five published studies which examined whether post-measles infection is associated with long-term excess mortality (Table 2), there is a trend towards lower subsequent mortality for individuals who survived acute measles infection (RR_{random effect}=0.49 (0.26-0.91)). Second, the hypothesis does not explain that the beneficial NSEs have been found in many situations where there was no measles infection and where there could not have been any prevention of long-term measles-induced memory loss[22, 36, 37, 39]. Thirdly, other live vaccines, for which there was little mortality to
prevent, such as oral polio vaccine (see below) and smallpox vaccine post-eradication[43], have also been observed to have beneficial NSEs. Fourthly, another live vaccine, BCG, has been shown to induce innate immune training resulting in reduced susceptibility to unrelated pathogens [4, 7]. Hence, the NSEs of MV are presumably to a large extent related to immune-training mechanisms[1].

In conclusion, triangulation of the randomized trials and observational studies provided evidence for beneficial NSEs of MV. It would be impossible to account for these data patterns without recognizing that MV have beneficial effects, which cannot be explained by the prevention of measles infections. In other words, the alternative hypotheses that MV has only specific protective effect and no beneficial NSEs, or that all the estimates were biased, are not compatible with the data.

3.2. Example: Oral polio vaccine

OPV was not reviewed by WHO, and there is still no general acceptance that OPV has beneficial NSEs[9]. However, the following observations are important:

First, the only RCT of a birth dose of OPV (OPV0) in Bissau found a 17% (-13 to 39%) reduction in infant mortality [44]. Children in this trial received numerous OPV-campaign vaccinations during their first year of life and, if OPV has beneficial NSEs, such additional doses would lower mortality in both arms of the RCTs diminishing the measured difference. Analyzing the effect of randomization to OPV before the children received campaign-OPV, OPV0 vs. no OPV0 was associated with a 32% (0-55%) reduction in infant mortality[44].

Second, several studies have shown OPV-campaigns to be associated with a marked decline in the general mortality rate[45-47].
Third, OPV campaigns have modified estimated mortality outcomes in studies of other vaccines[45, 48].

Fourth, boosting with a second dose or further doses of campaign-OPV reduced mortality substantially[33, 46].

Fifth, several studies have shown that mortality is lower when OPV-only rather than the recommended combination of OPV and DTP has been given[49, 50].

Sixth, two studies of the introduction of DTP and OPV in the rural and urban areas of Guinea-Bissau showed that mortality was higher when DTP-only was administered rather than DTP and OPV simultaneously[50, 51]. In other words, OPV appears to have reduced the potential negative effects of DTP.

Seventh, most studies suggest the OPV has a stronger beneficial effect for boys than for girls[44-46].

Eight, OPV was recommended at 2, 3 and 4 years of age in Denmark until 2001, and receiving OPV was associated with a 27% reduction in hospital admissions for respiratory infections[52].

Ninth, in RCTs of OPV vs. inactivated polio vaccine (IPV), OPV was associated with less diarrhea in Bangladesh[53] and less otitis media in Finland[54].

Hence, the triangulation of these results from the RCT and observational studies indicates that OPV has beneficial NSEs. The alternative hypothesis, that OPV has only specific protective effect and no beneficial NSEs, is contradicted by several trends that cannot be explained by biased estimates.
3.3. **Summarizing the importance of triangulation**

An RCT typically studies the effects of getting one vaccine vs. not getting it, and face the ethical challenges and the challenges related to interfering by effect-modifying health interventions. The examples above illustrate how triangulation of RCTs and observational studies, attacking the question whether a given routinely used vaccine is likely to have NSEs from several different angles, may provide compelling evidence for such effects.

Clearly, each of the different observational studies suffer from potential bias. Importantly, however, the direction of the bias varies from study to study. For some biases, the direction are in different directions. For instance, we know that healthy vaccinee bias - the fact that it is the healthy children, who are brought for vaccination first - is an important source of bias in observational studies, and it will lead to favorable estimates of the vaccine under study. Therefore, it is important to note that in the example of OPV, receiving OPV alone rather than OPV+DTP as recommended, was associated with lower mortality. Furthermore, it is important to note that the comparisons of female-male mortality of both MV and OPV are not prone to healthy vaccine bias; in both examples sex-differences were found, which cannot be explained from the perspective of the specific vaccine effects. Lastly, from the specific perspective, it should not modify the mortality effect of an intervention when another intervention is given; however, this was seen for both MV and OPV.

4. **Current state of affairs**

In spite of the consistent NSE patterns, which have been identified, it is still a major point of critique that most data related to NSEs is observational and therefore of poor quality and
potentially biased. Hence, the prevailing argument is that RCTs are needed to resolve specific controversial issue; for example, that the polarized views about the NSEs of DTP can only be resolved by RCTs[9]. Unfortunately, this is a catch-22, since it would not be considered ethical to delay DTP. Furthermore, RCTs with mortality as the outcome require very large sample sizes and thus are very expensive, they take too long to conduct, and with current funding levels they will never be able to address all the issues related to NSEs. Also, though more RCTs are definitely desirable, they are unlikely to resolve the numerous controversies related to the NSEs. One RCT in one specific contextual situation is not going to remove all the information from previous studies.

Almost 4 years after WHO reviewed the evidence for NSEs and recommended further research, IVIR-AC has now submitted for public comments two protocols of RCTs to measure the NSE impact of BCG and MV on child mortality (http://www.who.int/immunization/research/implementation/nse_protocol_comments/en/).

1) A BCG trial will compare mortality between 0 and 14 weeks of age for children randomized to BCG-at-birth plus routine vaccines at 6-14 weeks of age vs. placebo at birth and routine vaccines at 6-14 weeks, with BCG at 14 weeks of age.

2) An MV trial will compare mortality between 14 weeks and 2 years of age for children randomized to an additional dose of MV co-administered with DTP3 vs. placebo co-administered with DTP3.

Noteworthy, the two NSE trials do not compare BCG vs no BCG (placebo) or MV vs no MV (placebo) as other vaccines will be given with the intervention and/or during follow up. In contrast to the approach rightly emphasized in the WHO review - limiting follow-up to the time when a given vaccine was the most recent vaccine - the proposed trials will have a long follow-up period
during which several other vaccines are given, making it impossible to determine the effect of BCG alone or MV alone. In addition, the clinical interventions proposed in the BCG trial are so massive that the trial may well change the local cause-of-death structure. This implies that the RCT ends up measuring the possible NSEs in the frailest children with malformations, genetic defects, and HIV-infection rather than the possible NSEs against the common severe infections which kill most children in low-income countries. That is basically not the way to explore the importance of the potential NSEs of vaccines we already use or the vaccines we want to introduce in the global immunization program.

5. The way forward

To advance the understanding and use of the NSEs for improved human health, we need both observational studies and RCTs.

We need observational studies to establish the unexpected patterns, which might be worthwhile pursuing in RCTs; nearly all currently known NSEs patterns were first detected in observational studies before being tested in RCTs[22, 55]. To expand the knowledge of the NSEs of vaccines, we need real-life data of effects on survival from situations where there is minimal interference in term of preventing mortality in other ways, and minimal risk of bias. For example, it should be possible to use first, natural experiments, second, vaccination status among hospitalized patients to detect links to hospital case-fatality and causes of death, third, comparative male and female mortality rates in situations with limited sex-differential treatment, and fourth, analyses of changes in mortality rates before and after national vaccination campaigns and other interventions to establish such patterns (Box 1).
We also need RCTs to advance our knowledge. Unfortunately, the large RCTs of NSEs proposed by IVIR-AC have been defined in a manner so they are unlikely to lead to any certainty about the NSEs of MV and BCG or any policy changes. It might be more appropriate to focus on testing policy modifications related to potential NSEs of the currently used vaccines and the currently used vaccination schedule. We have proposed a number of such trials in Table 3. They would show how important NSEs might be in the current situation in high-mortality countries and they could translate directly into policy if relevant. Hence, ideally we should organize multicenter RCTs of whether such changes in ages of vaccination, practice of vaccination or in sequence of vaccination change mortality patterns for the affected age group. The planned phase out of OPV in favor of IPV for instance provides a unique possibility for assessing the NSEs of these vaccines.

With respect to the two examples given: Awaiting further proof-of-concept trials of whether MV and OPV have beneficial NSEs will only delay matters. The research agenda should be to find out how important these effects are in relation to specific vaccine schedules and if they can be used to improve the effects of these schedules. Immediate policy changes with emphasis on providing MV earlier than 9 months of age seem warranted, not least seen in the view that maternal immunity is declining, and many children now have no measurable maternal measles antibody left by 4-5 months of age[24-26]. In several analyses, DTP modified the effect of MV. Rather than the “certainty” of the beneficial NSEs of MV, focus should also be on what other factors might affect the NSEs of MV. For example, we have found that routine OPV, OPV-campaigns and vitamin A-campaigns may modulate the NSEs of MV[22, 25, 48].

It is usually argued that OPV has to be removed because it can cause paralytic polio in rare cases or revert to a more pathogenic form. However, if OPV reduces all-cause mortality, it may turn out to have been a poor decision for the poorest children with limited access to health care to remove
OPV in 2020 and substitute it with IPV. Hence, the research agenda would be to find out under what epidemiological and immunological conditions OPV’s beneficial effects would be enhanced or reduced/eliminated, and how they may be replaced if OPV is removed.

Science is about finding consistent patterns, which can be used to make deductions and increase predictability. From a public health perspective, the overall aim is to find causes of effects, which can be used in interventions and health policies. It should be immaterial whether such patterns are based on RCTs or not. While each single observational study may reach the wrong conclusions due to confounding, RCTs may also reach the wrong conclusion, not least due to unrecognized interactions, which may vary from one situation to another, or because they provided so much clinical care that the outcome is not representative for the planned target population.

Science is also about accounting for all data. Unfortunately, in the current “medical science” culture, there is a tendency to focus on RCTs as a method to control potential biases. This goes hand in hand with a tendency to dismiss observational studies, because they might have risk of bias, even when it would not be ethically acceptable to conduct an RCT. Studies are dismissed even though the potential bias has not been shown to have an effect or the bias is in the opposite direction. In this way, we throw away information, which may be important in a triangulation analysis. For example, it has not been possible to conduct RCTs of DTP in high-mortality areas, but all natural experiments and observational studies suggest that DTP is associated with increased female mortality[34]. Triangulation of these data actually provided an explanation of why HTMV was associated with increased female mortality[17]. An interpretation accounting for all data would therefore have to conclude that DTP most likely has deleterious NSEs for girls, and it would be indicated to try minimize or remove these negative effects. Hardcore RCT promoters dismissing
this interpretation because it has not been tested in RCTs would be doing a disservice to public health and the understanding of human health.

In conclusion, instead of focusing only on RCTs, results from both RCTs and non-RCT studies should be triangulated to strengthen the causal interpretation; it will be particularly important to cover possible interactions with other immune-stimulatory interventions.
6. Expert commentary

During the last decades, there has been an increasing focus on RCTs from the industry and policy makers. At the same time, among epidemiologist, advocates of the so-called “potential outcomes approach” have promoted the pre-eminence of the RCT for assessing causality; other study designs (i.e. observational studies) are only considered valid and relevant to the extent that they emulate RCTs[56]. I Observational studies are prone to bias, and unmeasured confounding is often invoked as an explanation for single study result— as there are always variables, which have not been covered – and thereby observational study findings, which contradict current understanding, easily risk being dismissed.

Almost all the currently used vaccines were introduced without studies testing their effect on overall mortality. The discovery that vaccines may have non-specific and sex-differential effects stems from the RCTs conducted on a new high-titre measles vaccine, which turned out to be associated with increased female all-cause mortality in spite of being protective against measles[57]. However, it has been and is ethically difficult to conduct RCTs of vaccines, which are already part of the vaccination program, because it often means withholding the vaccine from eligible children. Furthermore, as child mortality is fortunately declining globally, RCTs with mortality as the end-point become increasingly large and it is now only the largest stakeholders, like industry or WHO, with funding from global donors like Gates Foundation, which can afford to conduct such RCTs. If at the same time observational studies are only accepted as (weak) evidence as long as they emulate RCTs, then we have created a situation, where it is virtually impossible to document the existence of NSEs and to continuously monitor and evaluate the overall effect of health interventions.
Fortunately, there is now increasing acknowledgement among epidemiologist that observational data can, if appropriately triangulated, importantly strengthen the argument for causal inference[10, 18, 58]. Triangulation involves addressing a causal question by integrating results from several different approaches that have different and unrelated key sources of potential bias. If the results of different epidemiological approaches all point to the same conclusion, this is likely to be the correct answer, particularly when the key sources of bias of some of the approaches are in opposite directions.

During the last decade, there has been a large number of observational studies, which contradict that vaccines have only specific effects, and which have turned out to be repeatable and internally consistent. As stated by Cornfield in 1959: “if only one hypothesis can explain all the evidence, then the question is settled, even if the evidence is observational”[59].

Limiting the potential assessment of NSEs to RCTs will severely limit the tools for assessment. There are alternative study designs, which provide additional valuable information. We have used MV and OPV as examples, but the methods applied may obviously also be used for other vaccines. There is and there will continue to be, a huge demand for post-licensure assessment of vaccines in the real-life context, not least to evaluate their NSEs. We strongly advocate for these alternative study designs. In conclusion, in addition to RCTs, observational studies and triangulation can be used to assess the likelihood of NSEs of routinely used vaccines.
7. Five-year view

Vaccines are justified in terms of their effect on overall mortality and morbidity. Five years from now, we think it will be evident that simply testing a vaccine’s effect on the specific antibody response and/or protection against the target disease will not be sufficient. The increasing evidence for NSEs of vaccines from epidemiological studies - supported by evidence from immunological studies that vaccines indeed influence the immune response to non-related pathogens – will have made it clear that vaccines need to be evaluated for their effects on overall health. In addition, it is inherent for NSEs that there is no such thing as a context independent effect. Hence, one cannot assume that it is sufficient to document that a vaccine has beneficial effects on overall health in one context; the effect may change as other health interventions are introduced or re-scheduled.

Hence, we will need studies to assess the effect of already recommended and new vaccines on overall health, and we will need continued monitoring and evaluation of vaccines, and whether there are signals that their effect has changed when the context changes.

While this might sound very complex, there is no doubt that provided the observed NSEs are real, we can reduce child mortality and morbidity much more, merely using the existing health interventions in a smarter way.

Monitoring and evaluating vaccines for their overall health effect by means of RCTs is not feasible, nor rational for the reasons argued above. Thus, there will be a need for a standard tool-box of tailored but still flexible study designs and methodologies aimed at evaluating, monitoring and updating information about overall health effects of vaccines. Triangulation of RCTs and
observational study designs seems the most promising tool, and in five years, we anticipate that
the methodology has become more mainstream and more standardized; vaccinology or other
public health research areas may even have found ways to quantify the triangulation arguments.

8. Key issues:

- Vaccines may have non-specific effects (NSEs), in addition to their disease-specific effects
- Many people advocate that randomized controlled trials (RCTs) are needed to confirm or
  refute NSEs.
- RCTs have numerous problems as a tool to evaluate overall health effects of vaccines,
  including NSEs: they may be ethically problematic to conduct, they are expensive, take
  years to conduct, and they are not able to deal with the fact that NSEs are inherently
  context dependent, because as immuno-modulatory intervention, their effect may be
  modified by other immune-modulatory interventions. Hence, the results of an RCT may
  even have stopped being relevant before the RCT is completed.
- We advocate that triangulation of RCTs and observational studies constitutes a valid tool to
  assess potential NSEs.
- We provide two examples related to measles vaccine and oral polio vaccine. By
  triangulating studies of e.g. vaccinated vs. unvaccinated for several different vaccines,
  studies of sex-differences in mortality for several different vaccines, and
  vaccinated/unvaccinated before/after other interventions, a coherent argument for
  beneficial NSEs of these two vaccines has been built.
- For the future assessment of the overall health effects of new vaccines as well as
  continuous monitoring of already recommended vaccines, we cannot rely merely on RCTs;
results from both RCTs studies and non-RCTs studies should be triangulated to strengthen the causal interpretation; it will be particularly important to cover possible interactions with other immune-stimulatory interventions.

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Declaration of Interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.
References

Reference annotations

* Of interest

** Of considerable interest


Immunity. Cell Host Microbe. 2018;23:89-100 e5.** Proof of concept that a vaccine can alter the course of a subsequent unrelated pathogen challenge in humans


This paper shows that having a live vaccine as the most recent vaccine is associated with reduced morbidity in a high-income setting.


Table 1. Relative risk of hospital admission for infectious diseases, overall and by disease group in relation to measles-mumps-rubella (MMR) vaccination status

<table>
<thead>
<tr>
<th>Country</th>
<th>Vaccine groups compared</th>
<th>Overall relative risk of hospital admissions</th>
<th>Respiratory infections</th>
<th>Gastrointestinal and other infections (Other)</th>
<th>Ratio of effects for Respiratory infections/Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark[36]</td>
<td>MMR vs DTaP-IPV-Hib3</td>
<td>0.86 (0.84-0.88)</td>
<td>0.84 (0.81-0.86)</td>
<td>0.93 (0.88-0.97)</td>
<td>0.90 (0.85-0.96)</td>
</tr>
<tr>
<td>USA[37]</td>
<td>Live (88% MMR) vs inactivated vaccines</td>
<td>0.50 (0.43-0.57)</td>
<td>0.43 (0.37-0.51)</td>
<td>0.87 (0.68-1.11)</td>
<td>0.49 (0.37-0.66)</td>
</tr>
<tr>
<td>Italy[38]</td>
<td>MMR vs no MMR</td>
<td>0.29 (0.25-0.34)</td>
<td>0.18 (0.07-0.48)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>The Netherlands[39, 40]</td>
<td>MMR+MenC vs DTaP-IPV-Hib-PCV4</td>
<td>0.40 (0.38-0.41)</td>
<td>0.37 (0.36-0.39)</td>
<td>0.66 (0.60-0.73)</td>
<td>0.56 (0.50-0.62)</td>
</tr>
<tr>
<td>Country, time, reference</td>
<td>Age group</td>
<td>Period after follow-up after measles infections</td>
<td>Groups being compared</td>
<td>Measles infected vs. non-measles infected (95%CI)</td>
<td>Comments</td>
</tr>
<tr>
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</tr>
<tr>
<td>Guinea-Bissau, 1979-80[60]</td>
<td>0-6 years</td>
<td>6-18 months</td>
<td>Previous measles inf. vs not vaccinated</td>
<td>0.62 (0.16-2.30)</td>
<td></td>
</tr>
<tr>
<td>Guinea-Bissau, 1988[61]</td>
<td>Under 3 years</td>
<td>1 to 60 months</td>
<td>Measles cases vs controls</td>
<td>0.50 (0.22-1.16)</td>
<td>Adjusted age, sex, immunization status</td>
</tr>
<tr>
<td>Senegal, 1983-1986[62]</td>
<td>0-9 years</td>
<td>1 to 48 months; censored Dec 1986</td>
<td>Unimmunized measles cases vs unvaccinated, uninfected controls</td>
<td>Index cases: 0.27 (0.09-0.85) Secondary cases: 1.10 (0.80-1.51)</td>
<td></td>
</tr>
<tr>
<td>Senegal, 1992-1996[63]</td>
<td>0-6 years</td>
<td>1-48 months, censored Dec 1996</td>
<td>Exposed with clinical or subclinical measles vs exposed uninfected children</td>
<td>0.20 (0.06-0.74)</td>
<td>Adjustment had no effect</td>
</tr>
<tr>
<td>Bangladesh, 1982-1985[28]</td>
<td>Under 5 years</td>
<td>3-12 months post measles</td>
<td>Measles cases vs uninfected controls</td>
<td>0.40 (0.16-0.98)</td>
<td>Adjusted age, sex, siblings, maternal education, area</td>
</tr>
<tr>
<td>All studies</td>
<td></td>
<td></td>
<td></td>
<td>0.49 (0.26-0.91)</td>
<td></td>
</tr>
<tr>
<td>Justification</td>
<td>Group 1 (intervention)</td>
<td>Group 2 (current practice)</td>
<td>Interpretation if Group 1 is associated with beneficial effects on morbidity and/or mortality</td>
<td></td>
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<td>------------------------------------------------</td>
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</tr>
<tr>
<td><strong>BCG vaccine</strong></td>
<td>No restrictive vial policy for BCG</td>
<td>Restrictive vial policy for BCG</td>
<td>Group 1 will get BCG earlier and since TB is a very rare cause of death in the first months of life, any observed benefits of BCG will be non-specific</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restrictive vial policy is practiced in many countries and delay BCG vaccination</td>
<td>Restrictive vial policy for BCG</td>
<td>Restrictive vial policy for BCG</td>
<td>If there is beneficial effect in homes without TB exposure this would suggest beneficial NSEs of BCG-revaccination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCG-revaccination of scar-negative children since BCG-scar is associated with better survival</td>
<td>Revaccinate scar-negative children</td>
<td>No revaccination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DTP vaccine</strong></td>
<td>BCG-revaccination with DTP3</td>
<td>Current practice: Only DTP3</td>
<td>A beneficial effect would show that DTP3-associated morbidity/mortality can be reduced. This could be due to a beneficial effect of BCG and/or that BCG modifies the negative effect of DTP3</td>
<td></td>
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</tr>
<tr>
<td>Give or not give DTP3 with MV to children who are missing DTP3 at the time of MV</td>
<td>Only MV (drop DTP3)</td>
<td>MV+DTP3</td>
<td>Group 1 will get MV alone; benefits would suggest negative NSEs of DTP3 with MV</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>OPV</strong></td>
<td></td>
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</tbody>
</table>
**OPV is about to be replaced with IPV**

| OPV schedule | IPV schedule | As polio infection does not contribute to mortality, beneficial effects would suggest that OPV is better for child survival than IPV. |

**Cluster-RCTs of OPV campaigns**

| OPV campaign | No campaign | Benefits would suggest that OPV has beneficial NSEs |

**MV**

| No restrictive vial policy for MV | Restrictive vial policy for MV | Group 1 will get MV earlier and since measles infection is unlikely to be widespread (can be monitored) benefits would suggest NSEs of MV |

**Restrictive vial policy is practiced in many countries and delay measles vaccination**

| Additional dose of MV 4-6 weeks after DTP3 | No additional MV | Group 1 will get MV earlier and since measles infection is unlikely to be widespread (can be monitored) benefits would suggest NSEs of MV |

**Cluster-RCTs of MV campaigns**

| MV campaign | No campaign | Benefits would suggest that MV has beneficial NSEs |
Figure 1: A typical vaccination program in a low-income country

*BCG = Bacille Calmette Guérin; DTP = diphtheria-tetanus-pertussis vaccine, increasingly given in combination with vaccines against *H. influenzae* and *Hepatitis B* as "pentavalent vaccine"; OPV = oral polio vaccine.
<table>
<thead>
<tr>
<th>Examples of study designs</th>
<th>Hints that vaccines have NSEs (reference gives example)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCTs: Earlier or delayed routine vaccines (golden standard)</td>
<td>Changes in overall mortality/morbidity rates which are not explained by the effect of the intervention vaccine on the target disease[55]</td>
</tr>
<tr>
<td>Natural experiments: Take advantage of national vaccination campaigns, shortages of vaccines, etc.</td>
<td>Changes in overall mortality/morbidity rates which are not explained by the effect of the “natural experiment vaccine” on the target disease[50]</td>
</tr>
<tr>
<td>Observational studies:</td>
<td></td>
</tr>
<tr>
<td>• Compare vaccinated vs. unvaccinated children for a given vaccines (inherent “healthy vaccinee bias” (HVB))</td>
<td>Increased overall mortality among vaccinated vs. unvaccinated children for a given vaccine (decreased overall mortality could to some extent be due to HVB)[64]</td>
</tr>
<tr>
<td>• Compare vaccinated vs. unvaccinated children for several given vaccines (can be used to compensate for HVB)</td>
<td>Changes in overall mortality among vaccinated vs. unvaccinated children from one vaccine to another vaccine (HVB would be expected to affect all vaccines, so of one vaccine is associated with more reduced mortality than another, and this cannot be explained by the effects on the target diseases, it is a sign of NSEs )[65]</td>
</tr>
<tr>
<td>• Assess female-male mortality among children vaccinated with a given vaccine (eliminates HVB)</td>
<td>In a context with no sex-preferential treatment and equal vaccination coverage, sex-differences in overall mortality among children vaccinated with the same vaccine points to NSEs[66]</td>
</tr>
<tr>
<td>• Compare female-male mortality among children with different vaccines (eliminates HVB)</td>
<td>Change in female-male mortality ratio with shift from one vaccine to another (should not occur)[67]</td>
</tr>
<tr>
<td>• Compare variations in sequence, e.g. in-sequence with out-of-sequence vaccines</td>
<td>It is particularly important if vaccinations out-of-sequence with the recommended schedule are associated with lower overall mortality (the opposite finding could be due to HVB)[68]</td>
</tr>
<tr>
<td>• Study effect of boosting with vaccines, for which boosters should have little mortality impact</td>
<td>Finding boosting effects points towards NSEs[33]</td>
</tr>
<tr>
<td>• Study effects of a vaccine in an RCT before/after a vaccination campaign</td>
<td>If a vaccine campaign modifies the effect of another vaccine tested in a randomized trial it would suggest NSEs[48]</td>
</tr>
</tbody>
</table>