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Correlates of vaccine-induced protection: methods and implications

Immunization, Vaccines and Biologicals

World Health Organization

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Abbreviations and Acronyms

BCG	Bacillus Calmette-Guerin (tuberculosis vaccine)
CFU	colony forming units
CI	(95% CI) confidence interval
CMI	cell-mediated immunity
CoR	correlate of risk (Qin et al. terminology)
E-IPV	enhanced inactivated polio vaccine
ELISA	enzyme-linked immunosorbent assay
EMEA	European Agency for the Evaluation of Medicinal Products
EPI	Expanded Programme on Immunization
FDA	Food and Drug Administration (USA)
FHA	filamentous haemagglutinin (*B. pertussis* antigen)
FIM	fimbriae (e.g. of *B. pertussis*)
GMC	geometric mean concentration
GMT	geometric mean titre
HbOC	*Haemophilus influenzae* type b oligosaccharide conjugate vaccine
HI	haemagglutination inhibition
Hib	*Haemophilus influenzae* type b
ICC	Intraclass correlation coefficient
ICH	International Conference on Harmonization
IFN-y	Interferon gamma
Ig	immunoglobulin
IM	immune (or immunological) marker
IPD	invasive pneumococcal disease
IPV	inactivated (killed) polio vaccine
LD_{50}	lethal dose, 50%
LTT	lymphocyte transformation test
MLSA	*Mycobacterium leprae* soluble antigen
Mtb	*Mycobacterium tuberculosis*

OMP(C)	outer membrane protein (complex)
OPA	opsonophagocytic antibodies
OPV	oral polio vaccine
PPD	purified protein derivative
PRN	plaque reduction neutralization (e.g. measles antibody assay)
PRP	polyribosyl ribitol phosphate (*H. influenzae* polysaccharide)
PT	pertussis toxin
PTE	proportion of treatment effect explained
PTN	pertactin (*B. pertussis* antigen)
RCT	randomized controlled trial
SBA	serum bactericidal antibody (rSBA: SBA with rabbit complement)
SD	standard deviation
SES	socioeconomic status
SoP	surrogate of protection (Qin et al. terminology)
VE	vaccine efficacy (or effectiveness), defined as $(1 - R_v/R_{nv})$ where R_v and R_{nv} are outcome risks in similarly exposed vaccinated and non-vaccinated individuals, respectively
VE_{CE}	vaccine efficacy (or effectiveness) based on clinical endpoint
VE_{IM}	Vaccine efficacy (or effectiveness) based on immunological endpoint
VZV	varicella-zoster virus
WHO	World Health Organization

1. Introduction

The ability to assess the protective efficacy of a vaccine by measuring the proportion of vaccinees who generate a particular immune response, without having to measure clinical outcomes, has significant advantages. The availability and quality of such substitute endpoints[1] are important for vaccine development, licensure and effectiveness monitoring. A better understanding of the interrelationships between vaccination, the immune response, protection, and clinical outcomes is thus of interest not only to regulatory authorities but also to microbiologists, immunologists, epidemiologists and statisticians.

This is a complex and controversial topic, and many aspects need clarification. Although regulatory bodies have drawn up definitions for "correlates" and "surrogates" of protection for the purpose of licensure, these terms are not used consistently among regulators, nor in the broader literature. Different study designs, each with their strengths and weaknesses, have been used to evaluate immunological substitute endpoints of vaccine-induced protection. Various statistical tools have been developed to evaluate these endpoints, but few epidemiologists are familiar with the details of these methods. Immunological substitute endpoints can be relative or absolute quantities, and further information is needed on how they are affected by factors such as the challenge dose, the mechanism of action of the vaccine, the environment, or host characteristics.

This document presents an overview of definitions and methods relating to studies of substitute endpoints. It aims to facilitate communication and to encourage the development of a broad research agenda on the issue. Although the greatest interest in this topic currently relates to vaccines, immunological correlates of protection have far-reaching implications for passive protection (maternal immunity and immunoglobulin prophylaxis), risk screening (e.g. tuberculin or rubella antibody testing in pregnant women) as well as for a basic understanding of pathogenesis and immunity.

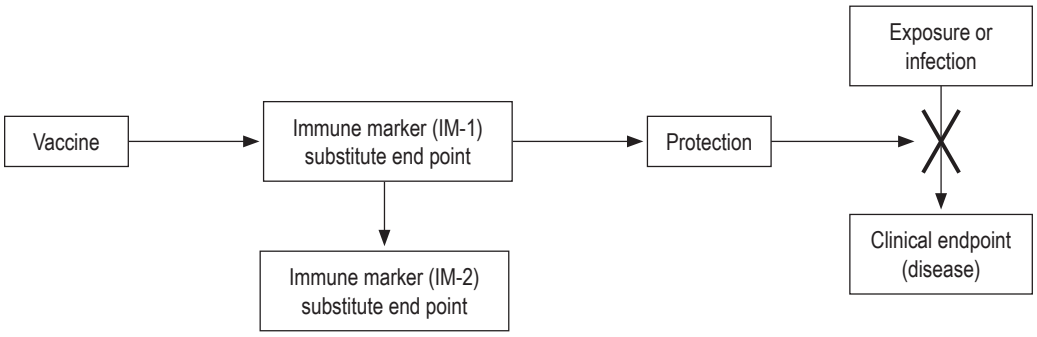
1.1 Definitions

Substitute endpoints have been discussed widely in the epidemiological literature in general and for vaccines in particular. Terms used include *correlate of protection, surrogate of protection, immune marker of protection, correlate of risk, proximal endpoint, distal endpoint, intermediate endpoint, protection endpoint,* and *clinical endpoint*. Authors and regulatory agencies use these terms with different definitions and nuances, associated with particular contexts. Annex 1 summarizes the major usage of terminology in the literature, a simplified schema of which is presented in Figure 1.

[1] In this document, substitute endpoint is a general term including correlates and surrogates of protection, or "intermediate endpoints" – in other words, quantities that may be measured instead of the clinical endpoint (i.e. disease) of ultimate interest.

Vaccines are given primarily to protect against disease, though protection against infection and even infectiousness may also be important effects. The implication of different clinical endpoints (infection, infectiousness, disease) are discussed later in this document, but we will, for simplicity, refer in general to protection against disease. Protection is evident, and measured, in terms of reduction in risk of the clinical endpoint of interest. Thus, by convention the (protective) efficacy of a vaccine (VE) is defined as the percent reduction in risk of (for example) disease amongst vaccinated individuals compared to equally exposed unvaccinated individuals, or $VE = (Rnv - Rv)/(Rnv)$, where Rnv and Rv represent incidence risk or rate of disease in non-vaccinees and in vaccinnees respectively. For many reasons (cost, time, ethics…), the enumeration of clinical endpoints is difficult, and thus there is a motive to find and use simpler substitute (eg. immunological) endpoints in order to evaluate vaccines.

Figure 1. Simple illustration of the induction of protective immunity by a vaccine



Arrows imply direct causal relationships

1.1.1 Correlates and surrogates

In Figure 1, protection is illustrated as the process which interrupts the pathway between "exposure or infection" and "clinical endpoint (eg. disease)". This is meant to cover both the prevention of infection among exposed ndividuals, and the prevention of disease among infected individuals. Distinguishing between these mechanism is often not possible.

IM-1 and IM-2 represent two sorts of intermediate or substitute immune markers involved in, or influenced by the relationship between vaccination and protection. As drawn, both IM-1 and IM-2 should change (i.e. appear, or increase in concentration) in the course of a vaccine's induction of protection. Both are thus *correlates* of protection, as they should be statistically associated with vaccine-induced protection. This definition of a correlate as an attribute that is statistically associated with an endpoint (without the association necessarily being causal) is consistent with widespread usage in the epidemiological and statistical literature *(1)*.

IM-1 is represented on the direct arrow line between vaccine and protection, indicating that it is on the direct causal pathway: the vaccine induces protection via a mechanism involving IM-1. Many authors restrict the word *surrogate* to what is represented by IM-1 in this diagram, and this paper follows their lead *(2–3)*.

IM-2, on the other hand, is not on the causal pathway between vaccination and protection – it represents something that happens as a consequence of being vaccinated, but which is not itself involved in protection. It is a correlate, but not a surrogate.

By this convention, all surrogates are correlates, but not all correlates are surrogates *(2)*. These are the senses used in this document.

Two qualifications are necessary. First, the distinction based on causal mechanisms is not always as simple as represented in Figure 1, and it is easier to establish a correlate than a surrogate. Second, other conventions are followed by prominent contributors to the literature and major regulatory agencies, who use the terms differently (see Annex 1). Until the subject matures appreciably beyond its current state, readers need to be aware of the variety of usages of the terms, and authors need to clarify their terms carefully.

1.1.2 Protection

In the context of vaccines, *protection* implies an immunological mechanism to prevent or to reduce severity of infection or disease. The mechanism can involve both humoral and cellular arms of the immune system. Many aspects of these mechanisms are not yet understood. Protection is complex, not only in its mechanism, but also in its manifestation. It may be *complete*, such that a protected individual suffers no ill consequences whatsoever if exposed to infection. It may be *incomplete*, implying that the severity of the consequences of the disease is reduced. (The term *partial* protection is sometimes used for this state, but is also used in a different way for vaccines, as discussed in Section 6.1.2). Or it may be *situation-dependent*, related for example to the environment or to exposure dose. These nuances complicate the evaluation of vaccines either directly, with disease reduction outcomes, or through substitute immunological endpoints, be they correlates or surrogates. These issues are addressed later in this review. At first, however, we assume that protection is a binary variable – i.e. that individuals are either (completely) protected or not.

2. Implications of different immunological mechanisms

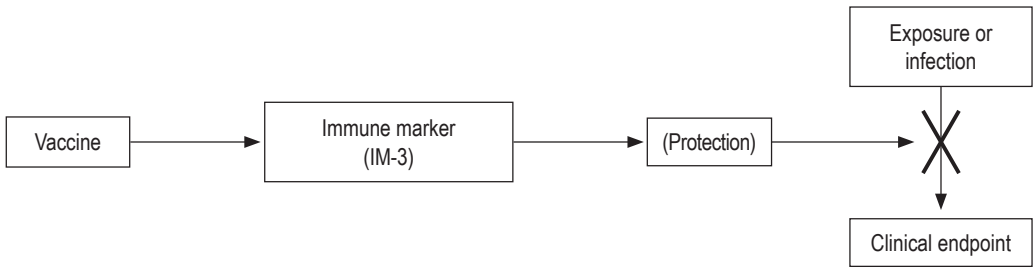
The validation and interpretation of immune markers as substitute endpoints for vaccine-induced protection depend on their relationships with the vaccine and with the clinical endpoint. Causal diagrams help to clarify these relationships, as shown below.

2.1 Single pathway with one immune marker

The simplest situation is a single pathway in which all the effect of the vaccine on the clinical endpoint is mediated through a single immune marker (IM-3), as in Figure 2.

The implication here is that the immune marker is both *necessary and sufficient* for the vaccine to provide protection. Such a marker satisfies the strictest criteria for a *surrogate*.

Figure 2. Single pathway with one immune marker between vaccine and clinical endpoint



An example of such a single pathway is seen in data relating to antibody to influenza Weiss strain A as an immune marker for protection against hospitalization with influenza (described more fully in Section 4.1.1 below) (4).

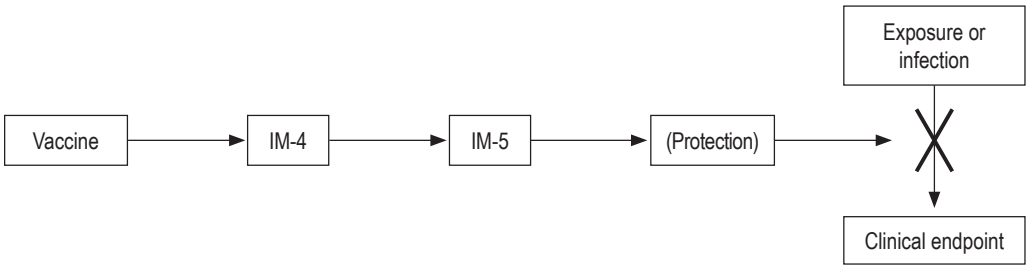
2.2 Two or more immune markers

If multiple markers are influenced by a vaccine, any one of them may or may not be necessary or sufficient for protection. There are several possible circumstances.

(i) A series of immune markers fully explains the observed protective effect of a vaccine

If clinical protection occurs only when a combination of immune markers is present and not when any element of the combination is missing, this suggests that all elements are necessary for protection as successive steps of a single causal pathway, as in Figure 3. An example of this could be a cytokine cascade that is initiated in response to vaccination (except that cytokines are not specific for particular infections). As each marker is necessary, provided it is specific to a particular infection, it would satisfy the strict criterion for a surrogate.

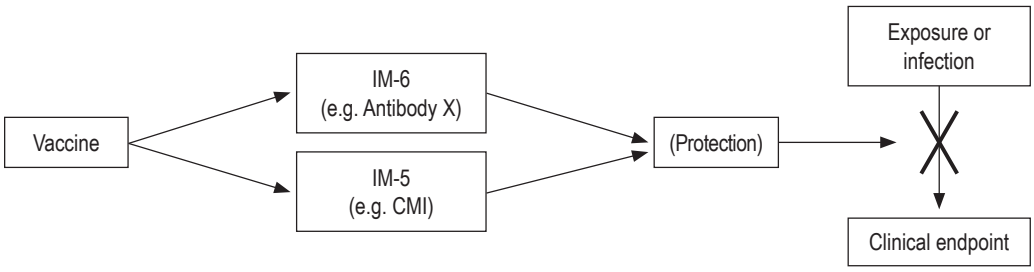
Figure 3. Single pathway with several immune markers



(ii) Immune markers are protective independently of one another

If clinical protection occurs when either one or another marker is present, this suggests that the markers belong to distinct pathways, as in Figure 4. Either immune marker can be sufficient for protection, but one may be more important than the other.

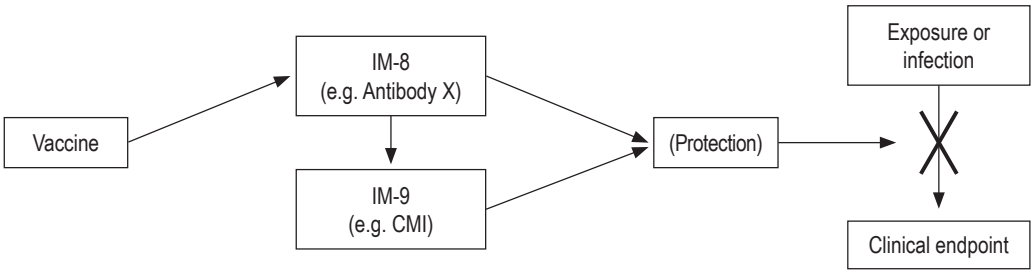
Figure 4. Multiple immune response pathways



CMI, cell-mediated immunity

Variations on this theme are possible, as illustrated in Figure 5.

Figure 5. Multiple immune response pathways between vaccine and clinical endpoint



EXAMPLE

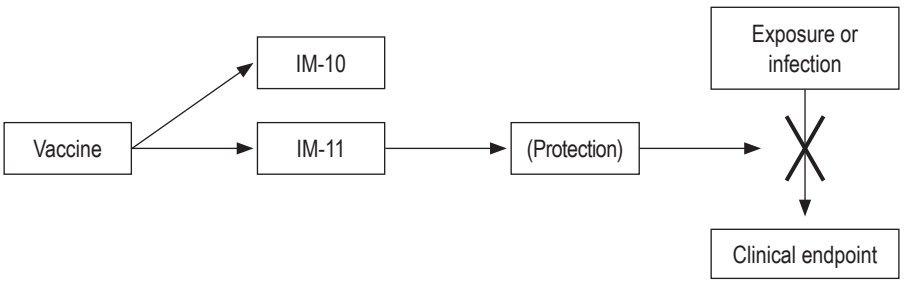
Several studies provide evidence that specific serum neutralizing antibodies are protective against measles. For example, maternal antibody protects against measles, and a study by Chen et al. (5) helped to derive a level of antibody associated with protection against typical clinical measles at the individual level (PRN >120 mIU/mL). However, other studies have shown that patients with agammaglobulinaemia can clear measles infection, whereas individuals with certain cellular immune deficiencies may develop severe disease. This is indirect evidence of the importance of cell-mediated immunity in protection against measles. Further evidence for the involvement of multiple pathways is added by the good correlation between antibody and lymphoproliferative response in vaccinated patients (6).

An important issue with multiple causal pathways is how to apportion the protection to each mechanism (as discussed below). Also, one of the pathways may not be observed or may be difficult to measure. The validation of such a hidden "marker" as a substitute endpoint can be challenging.

(iii) At least one immune marker has no role in protection

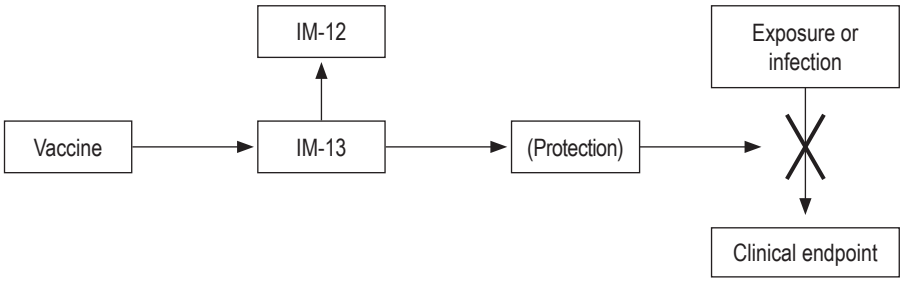
There are situations in which an immune marker is affected by a vaccine, e.g. it may be a collateral effect of the vaccine, but may be completely unrelated to protection or the clinical endpoint (Figure 6).

Figure 6. Immune marker as a collateral effect of the vaccine but unrelated to protection



Another possible mechanism is one in which a marker (Figure 7, IM-12) is not protective against the clinical endpoint, but is associated with a marker on the protective pathway induced by the vaccine (IM-13).

Figure 7. Immune marker induced by a step on the causal pathway but not involved in protection



IM-13 may or may not be observed. If IM-12 is a necessary product of IM-13, and if the association between IM-12 and IM-13 is strong, there will be a statistical association between IM-12 and the vaccine and between IM-12 and the clinical endpoint. This means that IM-12 could accurately predict the vaccine's efficacy, and may be useful for vaccine development and licensure . Mere statistical validation of IM-12 as a substitute endpoint will fail to detect that IM-12 is not on the causal pathway. In contrast, if IM-12 is not a necessary product of IM-13 or their association is not strong (e.g. there is considerable variability between individuals), IM-12 may be only weakly correlated with vaccine efficacy (VE).

EXAMPLE

It is widely accepted that BCG-induced protection against tuberculosis is via cell-mediated immune mechanisms (corresponding to IM-11 or IM-13 in Figures 6 and 7). There has been some interest in whether the presence or size of a BCG vaccine-induced scar might be related to the degree of vaccine-induced protection against pulmonary tuberculosis (7). In this context, it is unclear whether the scar reflects only a local response to the vaccine (analogous to IM-10 in Figure 6), or whether the scar is a collateral product of the protective immune response induced by the vaccine (i.e. analogous to IM-12 in Figure 7).

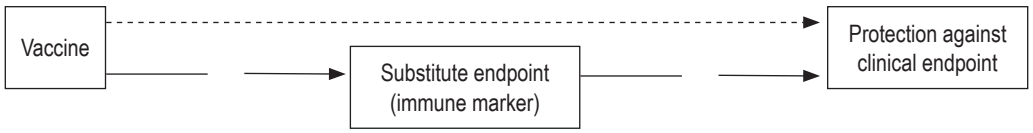
The complete chains of causality and interrelationships between vaccination, immune responses, protection and clinical endpoints are likely to be considerably more complex than the scenarios described above for most vaccines. New models continue to be developed. For example, a recent study by Kester et al. analysed combined humoral and cellular immune readouts to assess the independent and combined effects of T cell and antibody responses on protection against malaria (8). Drawing up causal diagrams allows a summary of the current stage of knowledge about these interrelationships and informs how the data may be analysed, using causal modelling or other techniques (9).

3. Study designs to evaluate vaccine-induced substitute endpoints

A variety of approaches have been used to identify, confirm and evaluate immunological markers as indicators of vaccine-induced protection. It is important to appreciate the logical arguments inherent in these designs, as they determine the nature and strength of the evidence. They are reviewed briefly below, with examples of their use.

To facilitate this discussion, a very simple diagram (Figure 8) depicts a situation in which a vaccine's effect is mediated entirely through a single substitute endpoint (immune marker). The effect of the vaccine on the clinical endpoint is represented by the dotted arrow, and the associations between the vaccine and substitute endpoint and between the substitute endpoint and clinical endpoint are represented by solid arrows [a] and [b], respectively.

Figure 8. Simplest possible relationship between vaccine, substitute endpoint and clinical endpoint



3.1 Trials and other experimental designs

Many investigators have allocated vaccines or immune globulins to humans or animals, and followed them up to assess either the immune response to vaccination and/or incidence of the clinical disease, compared to a control group who were not given the vaccine or globulins. Such designs allow the tightest possible control of potential confounding factors.

There are several studies in which results derived in animal experiments have been extrapolated to humans *(10–11)*. However, results from animal models are not always directly applicable to humans. For instance, serum IgA is actively transported into bile in the mouse model of rotavirus infection, but not in humans; thus protection against rotavirus in mice may not reflect the mechanism in humans *(12)*.

3.1.1 Randomized controlled trials with clinical endpoints

Randomized controlled trials (RCT) provide the ideal context to assess all elements of Figure 8: vaccine efficacy, the association between vaccination and immune markers (step a) and the association between markers, protection and clinical endpoints (step b).

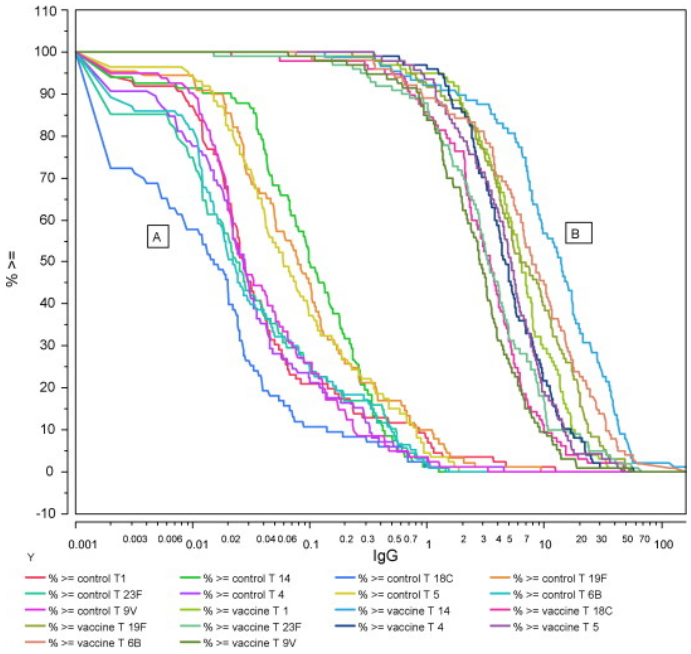
EXAMPLES

Santosham et al. (1991) carried out an RCT among infants to measure the efficacy of 2 doses of a *Haemophilus influenzae* type b (Hib) polysaccharide-OMP conjugate vaccine. Levels of serum antibody to Hib capsular polysaccharide were measured in all participants (2588 vaccinees and 2602 non-vaccinees) before the first dose of vaccine, at 2 months after the first dose, and at 2 and 8 months after the second dose. Infants were followed up for clinical disease with microbiological confirmation of *H. influenzae* infection. The authors analysed the associations between vaccination and clinical infection, observing 95% (95% CI: 72–99%) protection (the dotted arrow in Figure 8). As an antibody concentration of 0.15 μg/ml was thought to be protective (13), they noted that 45% infants had antibody concentrations above this prior to vaccination, rising to 90% at 2 months after the first and second doses, declining to 24% at 8 months after the second dose (reflecting step a in Figure 8). The only case among vaccinated children occurred at 15 months in a child whose antibody concentration was 1.49 μg/ml at 2 months after the second dose, but had declined to 0.14 μg/ml at 1 year of age, 3 months prior to illness (step b).

Participants in the Medical Research Council trial of BCG efficacy against tuberculosis (initiated in 1950) were negative to 100 IU tuberculin as an entry criterion to the trial (13 598 received BCG, 5817 received *M. microti* vaccine and 12 867 controls received nothing). All were skin tested 1 year after entry into the trial, giving data on 'step a', and followed up over the subsequent 20 years. Although BCG vaccination was shown to impart almost 80% protection against tuberculosis for the first 10 years after vaccination, this protection was not related to the level of vaccine-induced tuberculin reactivity (step b of Figure 8). The authors of the main report thus concluded "…with highly effective tuberculosis vaccines, the degree of protection conferred on the individual is independent of the tuberculin sensitivity induced by the vaccination" (14).

Saaka et al. (15) reported on a sub-study within a major trial of a 9-valent pneumococcal conjugate vaccine in the Gambia. The trial showed that the vaccine provided 77% protection against vaccine serotype-specific invasive pneumococcal disease (IPD), i.e. the dotted line in Figure 8. Blood was collected from 212 of 17 437 children enrolled and serum antibody levels compared between those allocated vaccine and those receiving the placebo (Figure 9). The geometric mean titres and proportion of infants with antibody levels above 0.2, 0.35 and 1.0 μg/ml were appreciably higher in the vaccine group compared to the placebo group (step a in Figure 8). Using the methods described here in Section 4.2.2, they estimated a geometric mean protective antibody titre for all 9 serotypes combined to be 2.3 μg/ml (95% CI: 1.0–5.0) (step b).

Figure 9. Reverse cumulative distribution of antibody titres to each vaccine serotype in children receiving placebo or three doses of 9-valent pneumococcal conjugate vaccine



A, placebo; B, pneumococcal conjugate vaccine

Source: Reprinted from Saaka et al. (15) with permission from Elsevier.

3.1.2 Immunogenicity studies

These are exemplified by phase II vaccine trials in which individuals receive (preferably at random) a vaccine of interest or a placebo or older formulation of the vaccine, and their immune responses compared. Immunogenicity studies measure step a in Figure 8 and are typically carried out either prior to a full clinical efficacy trial or as part of a "bridging" exercise to compare the immunogenicity of a new batch or product with that of a known efficacious product.

EXAMPLES

Meningococcal C conjugate vaccine was licensed in the United Kingdom as a result of phase II immunogenicity studies. These compared serum bactericidal assay titres induced by the new vaccine to those induced by a licensed serogroup C polysaccharide vaccine, which demonstrated direct evidence of efficacy and accepted correlates of protection (16). The studies used rabbit complement (rSBA) with the "gold standard" criterion for protection based on serum bactericidal assay titres using human complement (hSBA) (17). Classic studies by Goldschneider et al. had shown that hSBA titres of ≥4 indicate protection and that some individuals with titres below 4 are also protected (18). Paired sample studies using both rSBA and hSBA showed that 85% of individuals with rSBA <8 had hSBA <4, and 93% of those with rSBA titres ≥128 had hSBA titres ≥4. However, for those with rSBA titres between 8 and 128, protection could be assumed if the rSBA titres rose fourfold as a consequence of vaccination. These studies involved the measurement of each step in Figure 8.

Edelman et al. vaccinated 116 volunteers with an alum-adsorbed purified botulinum F toxoid by one of three different schedules − intramuscular and subcutaneous administration, and boosters − and followed them up over two years. Response was measured as mouse anti-toxin titres. As 100% of guinea pigs with titre greater than 0.04 IU/ml, and 50–100% of guinea pigs with titres over 0.01 IU/ml, survived a challenge with 105 LD50 of type F toxin, a titre of 0.02 IU/ml was considered protective in humans. Using this criterion, it was estimated that the new vaccine left 7–16% of volunteers "unprotected" after eight weeks, and that 33–42% were not protected after one year (10).

A WHO leprosy vaccine including BCG and killed leprosy bacilli was first evaluated immunologically in Norwegian nurses and later in Malawi. A dose-response relationship between the concentration of killed leprosy bacilli in the vaccine and induced skin test reactivity to soluble antigens extracted from *M. leprae* (MLSA) was used to select the vaccine dose for subsequent trials (step a in Figure 8) (19). Both BCG alone and BCG + killed *M. leprae* were found to impart approximately 50% protection against leprosy in Malawi (dotted line in Figure 8), and skin test sensitivity to *M. leprae* antigens was found to correlate negatively with leprosy incidence in a general population in Malawi. However, there was no direct evaluation of whether vaccine-induced sensitivity to MLSA was associated with protection (step b in Figure 8) (20–21).

3.1.3 Passive immunization studies

Susceptible animals or human volunteers may be administered specific immune globulins and then followed up to evaluate the rate of natural infection as a function of antibody titre, effectively measuring step b in Figure 8. An important issue in these studies is whether the (passive) antibody titre required to provide protection may be higher than the antibody level associated with protection following natural infection, because it is not "supported" by other elements of the natural immune response.

EXAMPLE

Santosham et al. and O'Reilly et al. (22–23, respectively) administered Hib immune globulin prophylaxis to patients with agammaglobulinaemia and to newborn infants, respectively. Their results indicated that a serum antibody level of 0.15 µg/mL protected against invasive Hib disease in the short term, and that a level of 1 µg/mL was protective in the long term.

3.1.4 Challenge studies

Animals or human volunteers with known natural or vaccine-derived levels of immune marker may be challenged with different doses of a pathogen, and their responses related to their pre-challenge immune status. Again, these studies measure step b of Figure 8.

EXAMPLES

0.01 IU/ml of tetanus anti-toxin, measured in a neutralization assay in mice or guinea pigs, has been considered a minimum level for protection against tetanus. This level was based originally upon experiments with guinea pigs challenged with live *C. tetani* spores and on experience with horses given homologous antisera after acute injury carrying high tetanus risk. Several reports indicate that the 0.01 IU/ml level may not always be sufficient in humans with deep or necrotic wounds in which spores may be sequestered from circulating anti-toxin (11).

Tacket et al. (24) randomly allocated 85 volunteers to vaccination with an attenuated live cholera vaccine or a placebo, and measured levels of serum vibriocidal antibodies and serum IgG anti-cholera toxin antibodies after 10 days. A total of 51 volunteers (28 vaccinated and 23 placebo) had their immune response reassessed after three months and were subsequently challenged with an oral dose of 105 CFU of virulent *Vibrio cholera* 01. Among the vaccinated individuals, no association was found between 10-day or pre-challenge vibriocidal antibody titre and protection against cholera diarrhoea.

3.2 Observational studies

This term is used in its conventional epidemiological sense, implying studies involving only passive observation of groups or populations, with no controlled intervention.

3.2.1 Cohort studies

In cohort studies, individuals may be classified on the basis of their vaccination status and followed up to measure the immune response as the outcome of interest (step a of Figure 8). Alternatively, subjects may be classified by their immune response status and followed up to compare the occurrence of the clinical endpoint (step b of Figure 8).

EXAMPLES

Chen et al. (5) took advantage of an outbreak of measles among university students which took place shortly after a blood drive. Measles antibody titres in 90 stored samples of the donated bloods were measured by plaque reduction neutralization (PRN) and enzyme immunoassay (EIA). Most of the students had received measles vaccine as children. Eight out of 9 individuals with pre-exposure PRN titres of <120 contracted clinical measles according to the Centers for Disease Control and Prevention (CDC) case definition, compared to none of the 71 individuals with PRN titres >120. Although individuals with PRN titres >120 did not contract classical measles, 26 (70%) of 37 with pre-exposure titres <1052 reported at least one symptom, compared to 11 (35%) of 35 individuals with titres >1052. These data have been cited widely to support a PRN of 120 as a correlate of protection against clinical measles.

Jack et al. (25) followed children vaccinated with the plasma-derived hepatitis B vaccine in the Gambia, and correlated their levels of the antibody with subsequent incidence of hepatitis B surface antigen carriage. The results suggested that reaching a peak antibody level of >10 IU/L in the year following vaccination was associated with protection against hepatitis B persistent infection over the subsequent seven years (Figure 8, step b).

3.2.2 Natural history studies

Analysis of the immune response after recovery from an infectious disease may give an indication of protective components of acquired immunity, in particular for diseases associated with long-lasting natural protective immunity such as measles, varicella and mumps.

EXAMPLE

Ndumbe et al. (26) measured varicella-zoster virus (VZV)-specific antibody levels and lymphocyte stimulation responses to VZV antigen in 23 individuals who had experienced chickenpox either 6–8 weeks (n=10) or more than 5 years (n=13) previously. The geometric mean antibody titres (measured using an immunofluorescence test) among the 10 individuals with recent chickenpox was 158 (SD: 9.2, range <8–2048) compared with a mean titre of 41.8 (SD: 2.9, range <8–128) among those with less recent chickenpox. For the lymphocyte transformation tests (LTT), the stimulation indices ranged from 4.3–14.1 for those with recent infection, and from 6.1–24.2 for those with earlier infection. Given that second episodes of chickenpox are rare, all of these antibody and LTT levels may be interpreted as sufficient for protection against chickenpox disease. As declining cell-mediated immunity to VZV is probably associated with clinical zoster, and all these subjects were clinically well, the lower levels may also be interpreted as correlates of protection against zooster.

3.2.3 Maternal–newborn studies

The low incidence of vaccine-preventable diseases in neonates provides evidence for the protective role of maternal antibodies (27–28). Measurement of these antibodies and their rate of decline can give information on step b in Figure 8. As with the passive immunization studies mentioned in Section 3.1.3, it may be that the level of passively acquired maternal antibody required for protection is higher than the level needed if antibodies are actively acquired, as maternal antibodies lack the contribution or "support" of elements of the immune system that are not transferred across the placenta, e.g. those dependent upon lymphocytes.

EXAMPLES

Cord bloods and repeat samples every two months were collected from 1087 infants born in Nairobi, Kenya and tested for HI measles titres. The infants were allocated to receive measles vaccine at 5, 6, 7, 8, 9 or 12 months of age. This allowed detailed monitoring of the loss of maternal antibody and revealed that only 1 of 13 children with a pre-vaccination titre of 1:6, and neither of 2 children with a pre-vaccination titre of 1:12 seroconverted as a result of measles vaccination (29). This provides a correlate of "protection" against measles vaccine virus infection.

Glass et al. (30) followed 93 breastfeeding mother–child pairs in order to investigate whether IgA antibodies against cholera toxin and lipopolysaccharide protect against colonization with *Vibrio cholerae 01* and disease. Mothers without diarrhoea were recruited from households with cholera patients. IgA antibody concentrations were measured in breast milk, and infants were followed for the occurrence of colonization or disease. The researchers found no difference in the mother's breast-milk antibody concentration between colonized and non-colonized children, but the antibody concentration in breast milk was higher in colonized children who did not develop diarrhoea compared to those who did. This suggests that these IgA antibodies may protect against disease but not colonization.

3.2.4 Case–control studies

The case–control approach has been used to compare levels of immune markers prior to disease among individuals who did or did not develop the clinical outcome of interest (step b of Figure 8). The blood samples must have been collected prior to disease onset, and preferably prior to the exposure to infection. This often implies the sampling and testing of stored blood samples.

EXAMPLE

During an RCT of acellular pertussis vaccine in Sweden (31), serum samples were collected from children 21–77 days after vaccination and stored. These samples were later used in a nested case–control study of 126 children exposed to pertussis in their household. Children who developed pertussis (cases) were found to have had lower pertussis toxin (PT) IgG antibody levels in their stored samples than children who did not develop pertussis (controls): median concentrations among cases were respectively 79 U/ml for those with severe disease and 156 U/ml for those with mild disease, compared to a median concentration of 246 U/ml among controls (who contracted no disease). IgG to pertussis toxin thus correlates with protection in this context – but a precise level to use as a correlate cannot be specified. The combined effects of PT and other pertussis antibodies in predicting protection against pertussis disease are discussed in Section 4.1.2.

3.2.5 Ecological studies

In ecological studies, associations are drawn between vaccination status, the substitute endpoint (e.g. antibody titre) and the clinical endpoint only at the population level. It is therefore not possible to draw conclusions about the associations of interest at the individual level.

EXAMPLE

The efficacy of BCG against pulmonary tuberculosis has been shown to be lower in populations from Malawi than those from the United Kingdom. A series of studies compared data from the two countries to understand this difference, and to investigate potential substitute endpoints for protection (32–35). It was shown that pre-vaccination IFN-y response to PPD-Mtb (used as an indicator of T cell immune response) was well correlated to delayed-type hypersensitivity to *M. tuberculosis* antigens in both populations (32-33). It was also found that unvaccinated adolescents in Malawi had higher IFN-y responses than unvaccinated British individuals. Because of this difference in background level prior to vaccination, the increase in immune response after vaccination with BCG is higher in the United Kingdom than in Malawi (34). These studies did not demonstrate that any particular IFN-y response was a correlate of vaccine-induced protection against tuberculosis, but that the vaccine-associated *change* in IFN-y correlated with the vaccine-associated protection observed in the two populations.

4. Statistical methods for evaluation of substitute endpoints

Many of the examples above refer to correlations between one or more immune markers and degree protection. But statistical correlations are not helpful unless they are strong, and ideally reveal some clear criterion indicating protection. Several authors have addressed the statistical issues involved in the evaluation of interventions through substitute endpoints. Much of this literature is drawn from non-vaccine fields, such as cardiovascular disease or cancer risks and treatment effects (36). In the context of vaccines, several sorts of questions have been discussed:

i) How to make the step from demonstration of *correlation* between a substitute endpoint and protection, to identification of a useful *correlate*?

ii) How to distinguish between a *correlate* and a *surrogate* of protection (using the causal pathway criterion)?

iii) If an immune marker is a valid substitute endpoint in individuals, what is the relationship between the distribution of vaccine-induced titre(s) of the marker in populations and VE?

4.1 General methods for validating immunological markers as substitute endpoints for clinical protection

The demonstration that a vaccine induces an apparently "specific" immune response (Figure 8 step a) is not proof that the vaccine is protective. Similarly, the fact that an immune response is statistically associated with the risk of the clinical endpoint (Figure 8 step b) does not mean that it is responsible for the protection, or that it can predict the protective effect of the vaccine. Further criteria are needed.

4.1.1 The Prentice criteria

A key concept was introduced by Prentice in 1989 (3) in the context of a hypothesis-testing approach to the validation of substitute endpoints using RCT data. His four criteria for validation of a surrogate endpoint can be adapted for vaccine trials as follows:

i) Protection against the clinical endpoint is significantly related to having received the vaccine (the dotted line in Figure 8);

ii) The substitute endpoint is significantly related to the vaccination status (step a in Figure 8);

iii) The substitute endpoint is significantly related to protection against the clinical endpoint (step b in Figure 8);

iv) The full effect of the vaccine on the frequency of the clinical endpoint is explained by the substitute endpoint, as it lies on the sole causal pathway.

If the full effect of the vaccine is mediated via the immune marker (criterion iv), the incidence of the clinical endpoint should be the same among vaccinated and unvaccinated individuals at any particular titre of the immune marker, and the protective effect of the vaccine should disappear after adjusting for immune marker titre (e.g. in an individual level regression model).

EXAMPLE

Qin et al. (4) used data from a trial of a trivalent influenza vaccine carried out in 1943 and reported by Salk et al. (37) to illustrate the validation of influenza-specific antibody titre as a surrogate of protection for hospitalization with influenza (the clinical endpoint), using the Prentice criteria. VE of 73% was reported against hospitalization with influenza Weiss strain A (criterion i). Antibody titres to Weiss strain A were higher in vaccinated compared to unvaccinated individuals (criterion ii). The antibody titres were inversely associated with hospitalization for influenza (criterion iii). The risk of hospitalization was almost identical in vaccinated and unvaccinated individuals after adjusting for antibody titre (criterion iv).

The Prentice criteria were originally proposed for the statistical validation of substitute endpoints in the context of randomized controlled clinical trials. Schatzkin et al. (38-39) pointed out that this method could also be applied to observational studies.

The criteria are based on statistical associations, and criticisms of the criteria include the following.

- As Prentice acknowledged, the requirement that the substitute endpoint explains all the effect of the intervention (e.g. vaccination) on the clinical endpoint is restrictive. For many vaccines, it is unlikely that a single causal pathway leads from vaccination to clinical protection.
- Validation depends on showing that the incidence of the clinical endpoint in vaccinated and unvaccinated individuals is the same within each stratum of immune marker titre. Thus the regression model should include all subject characteristics that may predictboth the immune response endpoint and the disease/infection endpoint (40-41). The immune marker is measured after randomization and is itself affected by the intervention (vaccination). Thus the subgroups of individuals in each immune marker category are selected after randomization and the approach is susceptible to *post-randomization selection bias* insofar as the precise titre is influenced by factors other than the vaccine, such as age or host genetics (42). For example, a particular vaccine may be ineffective in young children (it does not induce a strong antibody response) but highly effective among older children (inducing high antibody titres), and younger children in a trial population may be at higher risk of disease than older children because they mix more and have higher intensity of exposure. In this scenario, vaccinated individuals with low antibody titres will include a higher proportion of young children than unvaccinated individuals with the same low titres, and this will result in different probabilities of disease in vaccinated and unvaccinated children due to the different age profile.

- There needs to be sufficient variability in the immune response among the unvaccinated individuals to allow comparison. For example, some of the unvaccinated need to have acquired the immune marker from previous exposure to the pathogen or related antigens. Validation of a surrogate using the Prentice method is not possible if the immune response being assessed occurs only after vaccination, e.g. if the response is not induced by natural exposure to the same or related antigens. (43)
- The Prentice criteria cannot be used to "prove" that the surrogate explains all the effect of a vaccine. This is because, in the adjusted model, it can never be shown that the relative risk associated with vaccination becomes exactly one (i.e. has no effect) after adjustment for precise antibody titre, only that the data are compatible with such a result.

4.1.2 Proportion of treatment effect

Adaptations have been made to the Prentice criteria in order to evaluate immune markers as substitute endpoints when there is more than one causal pathway between vaccination and the clinical endpoint of interest. In this circumstance, the question arises as to the *proportion* of the observed protection that can be explained by one or more of the pathways or markers. This general problem in epidemiology is sometimes described in terms of estimating the proportion of a treatment effect (PTE) explained by a marker (44). At its simplest, the PTE can be described as $(\beta-\beta_a)/\beta$, in which β is the unadjusted effect of vaccination on the clinical endpoint and β_a is the effect adjusted for the immune marker (45).

EXAMPLE

Storsaeter et al. (46) used the combined effect of pertussis antibodies in a logistic regression model to predict the risk of pertussis disease. They showed that IgG antibodies against three pertussis antigens (pertussis toxin, fimbriae and pertactin) were correlated with protection against clinical pertussis following household exposure (criterion iii of Prentice). Kohberger et al. (47) validated Storsaeter's model using the Prentice criteria, demonstrating that protection against pertussis was significantly related to vaccination (criterion i) and that vaccination was significantly related to pertussis antibody status (criterion ii). After investigating criterion iv of Prentice, they concluded that the combined antibody response explained 93% (95% CI: 51–100%) of the effect of the vaccine.

Concerns raised about the PTE method include the fact that it may not be a proportion (i.e. it may come out to less than 0 or more than 1) under certain circumstances (45). Participants at a 2001 National Institutes of Health workshop (reported by De Gruttola et al., 48) cautioned against the use of the PTE approach to assess surrogate endpoints in clinical trials, citing studies that demonstrated the difficulty of interpreting the PTE.

4.1.3 The Qin framework

Qin et al. (4) proposed a hierarchical framework to assess the validity of immunological markers as substitute endpoints, which distinguishes three different levels of association, termed as follows:

i) Correlate of risk;

ii) Level 1 "specific" surrogate of protection, divided into "statistical" and "principal" surrogates;

iii) Level 2 "general" surrogate of protection.

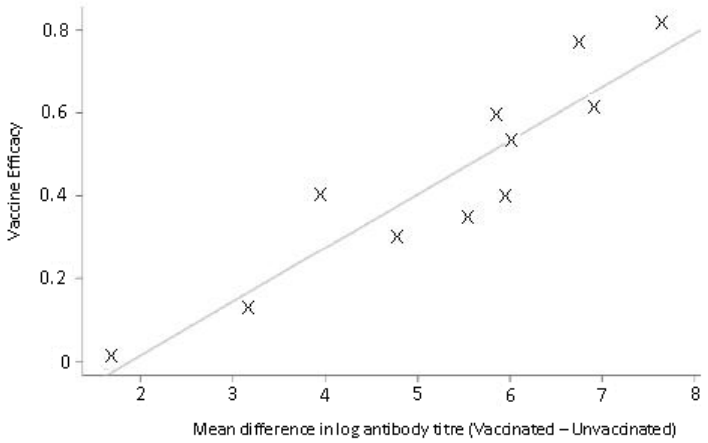
Statistical methods for applying the framework were reported by Gilbert et al. (40). As noted in Annex 1, Qin et al. define a correlate of risk (CoR) as an immune response that is associated with protection against a clinical outcome, whereas a surrogate of protection (SoP) is a CoR that predicts accurately the level of VE. Their definition of a correlate raises little difficulty, but their perspective on surrogates is more challenging and differs from the causality criteria suggested by other authors and outlined in Section 1.1. The division into level 1 and level 2 reflects whether the data relate to single or multiple populations, respectively.

The level 1 (specific) SoP is defined as "an immunological measurement that is a CoR within a defined population of vaccinees and is predictive of [vaccine efficacy] VE in the same setting as the trial". These surrogates are in turn subdivided into two categories ("statistical" and "principal") according to the logic of their validation:

- A level 1 "statistical" SoP is an endpoint that satisfies the Prentice criteria.
- A level 1 "principal" SoP is defined using a particular framework of causal inference, based on earlier methodological work such as that by Frangakis et al. (49), Rubin (50), and Follmann (51). This framework aims to address post-randomization selection bias found with the validation using the Prentice criteria, by estimating what the vaccine responses would have been had the non-vaccinated group been vaccinated. Follmann (51) and Qin et al. (4) suggest two methods to estimate these responses: (a) including a *baseline irrelevant predictor* (defined as a variable that correlates with measured levels of the immune marker but is not itself predictive of disease given the immune response), or (b) vaccinating a subset of uninfected controls at the end of the trial to see what their responses are (although these controls are themselves a selected group in that they did not develop disease during the trial). The logic of this approach is explained in greater depth in the paper by Gilbert et al (40) and in the commentary by Gilbert et al (41).

The level 2 "general" SoP is a surrogate that has been shown to predict VE across a range of different populations and settings, and can thus be used to assess VE in untested populations. A meta-analytical approach has been proposed to evaluate level 2 surrogates, using data collected from multiple trials. For each trial, the observed VE is plotted against measured differences in the immune response between vaccinated and unvaccinated individuals (Figure 10). The resulting relationship can be used to estimate VE in a new setting, based on the immune responses measured in that setting. This approach is data intensive (each study is a single data point) and extrapolation may not always be appropriate.

Figure 10. Level 2 surrogate of protection



Source: adapted from Qin et al. (4).

4.2 Methods for relating vaccine efficacy to measured levels of vaccine-induced immune markers

The approach used to validate a level 2 SoP in Qin's framework is one way to analyse the relationship between a substitute immune marker (for example, the mean difference in titres between vaccinees and non-vaccinees) and clinical protection by a vaccine. Other methods to do this can be divided into two main categories: threshold and continuous methods (Sections 4.2.2 and 4.2.3).

This Section begins by considering the distributions of immune markers in populations: their relationship to vaccination and their implications for protection. How these distributions can be harnessed to estimate VE is then discussed.

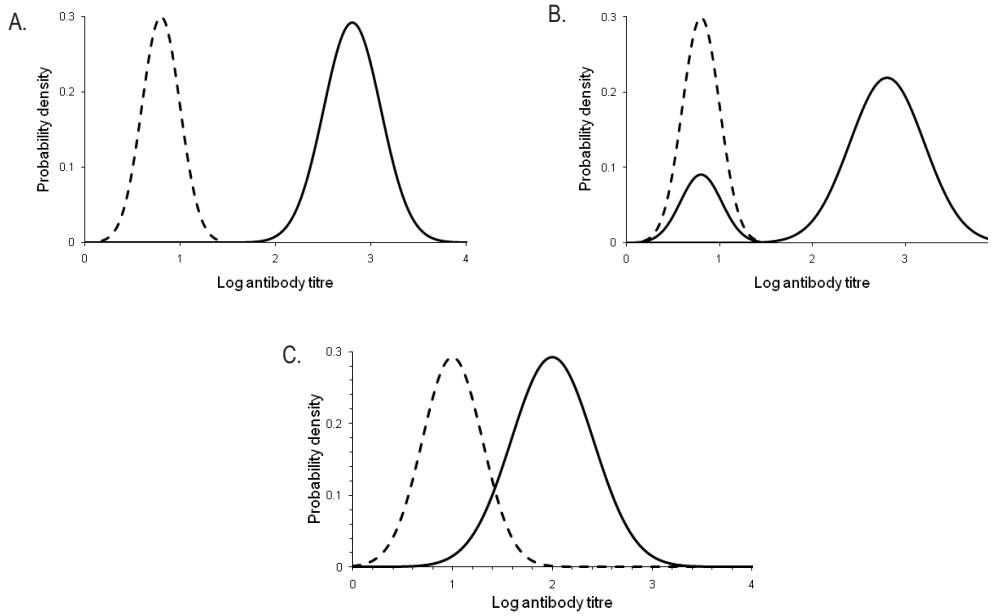
The methods discussed in this Section implicitly assume that the Prentice criteria are fullfilled, in particular that the relationship between the immune marker and the protection is the same in the placebo and the vaccinated groups. These methods are therefore susceptible to the criticisms associated with the Prentice criteria discussed in Section 4.1.1; for example the requirement for some variability in the immune response among the unvaccinated (placebo) groups. As highlighted in Section 4.1.3. Qin et al. (4) propose alternative methods within their framework that can be used to evaluate a potential immune marker in situations where there is no variability in the unvaccinated group.

4.2.1 Distribution of antibody titres and their implications

If a vaccine influences an immune marker, e.g. antibody titres to some particular antigen, this may be manifested in different population patterns. In some circumstances the distribution in non-vaccinees may be "low and narrow" – i.e. they may have very little response in assays for antibody to the antigen in question, and the distribution in the vaccinees may be clearly distinct, with little or no overlap between them (Figure 11A). In other circumstances there may be little antibody in the non-vaccinees, but the distribution in vaccinees may be bimodal, indicating that the vaccine "took in" (i.e. induced an immune response in) a proportion of vaccine recipients, but failed to

"take in" the remainder (Figure 11B). In a third circumstance the non-vaccinees may have a broad range of antibody titres, in which case the distribution of antibody response in vaccinees may end up with a greater or lesser overlap with that in the non-vaccinees (Figure 11C). These represent extremes of a spectrum of patterns which may occur with different vaccines and different immunological measures.

Figure 11.Three patterns of distribution of antibody titres in vaccinees and non-vaccinees

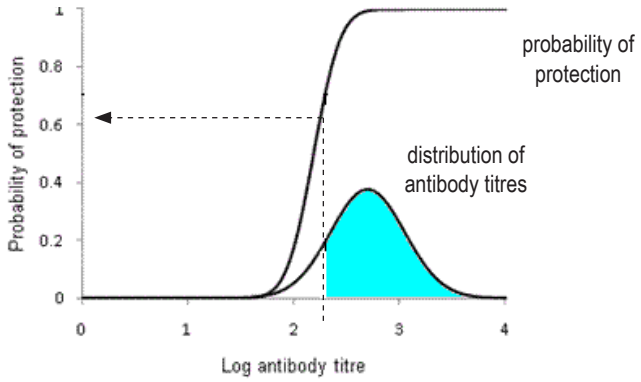


Solid lines, vacinees; dotted lines, non-vacinees.

Whatever the immune marker distributions among vaccinees and non-vaccinees, it is useful to consider them in relationship to protection, as illustrated in Figure 12. Here, a distribution of antibody titres is represented by the (lower line) frequency distribution, in this context ignoring whether or not individuals have been vaccinated or have a history of natural exposure. The upper cumulative frequency line represents the relationship between the immune marker and "protection". Assuming the Prentice criteria holds, individuals with less than a 1.5 log titre have little or no protection, whereas those with more than 3.0 logs have 100% protection.

In Figure 12, a log antibody titre of 2.3 is associated with 70% clinical protection – i.e. it reduces the risk of disease by 70% compared to individuals with less than 1.0 log antibody titre. What determines the variation in such antibody distributions and why a given titre may provide only 70% protection is discussed below.

Figure 12. Relationship between clinical protection and log antibody titre, and distribution of log antibody titres



The shaded area represents the proportion of individuals with antibody titres above a cut off of 2.3, who have at least a 70% probability of protection against the clinical endpoint.

Source: adapted from Nauta et al. (52).

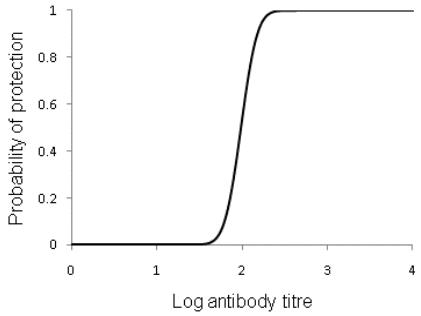
Nauta et al. (52), using influenza vaccination for illustration, showed that the relationship between antibody titres and clinical protection in a population depends not only on the mean but also on the standard deviation of the log-transformed antibody titres in that population. As a consequence, both parameters need to be considered when generalizing findings from a single population.

The ideas from Figures 11 and 12 can be used to evaluate vaccine efficacy.

4.2.2 Threshold methods

Threshold methods assume a step function: that there is a simple threshold level of the immune marker above which individuals are fully protected against the clinical endpoint and below which they remain fully susceptible (Figure 13). Consistent with the Prentice criteria, they also assume that this threshold is similar in vaccinated and unvaccinated individuals.

Figure 13. Hypothetical relationship between antibody titre and clinical protection with a simplifed 'step function'



Solid line, relation between antibody titre and clinical protection; dotted line, simplified step function. Source: adapted from Siber et al. (53).

The simplest way to estimate such a threshold is to relate pre-exposure immune marker titres to disease incidence in a cohort study and to adopt the titre above which no individual develops the clinical endpoint as the protective threshold. Thresholds derived using these methods are sometimes referred to as *individual-based correlates of protection* (54). An example of this is the study of Chen et al. (5) (Section 3.2.1) in which the surrogate of protection against measles, a PRN titre >120 mIU/mL, was derived by finding that nobody with an antibody titre above that threshold developed typical clinical measles during the outbreak studied. Other published examples of individual-based immunological markers of protection relate to diphtheria and rubella (55–56).

A second method uses the proportion of vaccinated and unvaccinated individuals below specified thresholds to estimate VE. The inference from correlate of protection to VE is based upon the traditional equation for observed clinical VE:

$$Equation\ 1:\ VE_{CE} = 1 - \frac{disease\ incidence\ in\ vaccinated}{disease\ incidence\ in\ unvaccinated}$$

VE, vaccine efficacy; CE, clinical efficacy;

Given that disease incidence should be a direct function of the proportion susceptible (assuming equal exposure), and assuming that a simple step function in the immune marker separates susceptibility and immunity, this translates directly into an equation for the immune marker-derived VE, here called VE_{IM} (53):

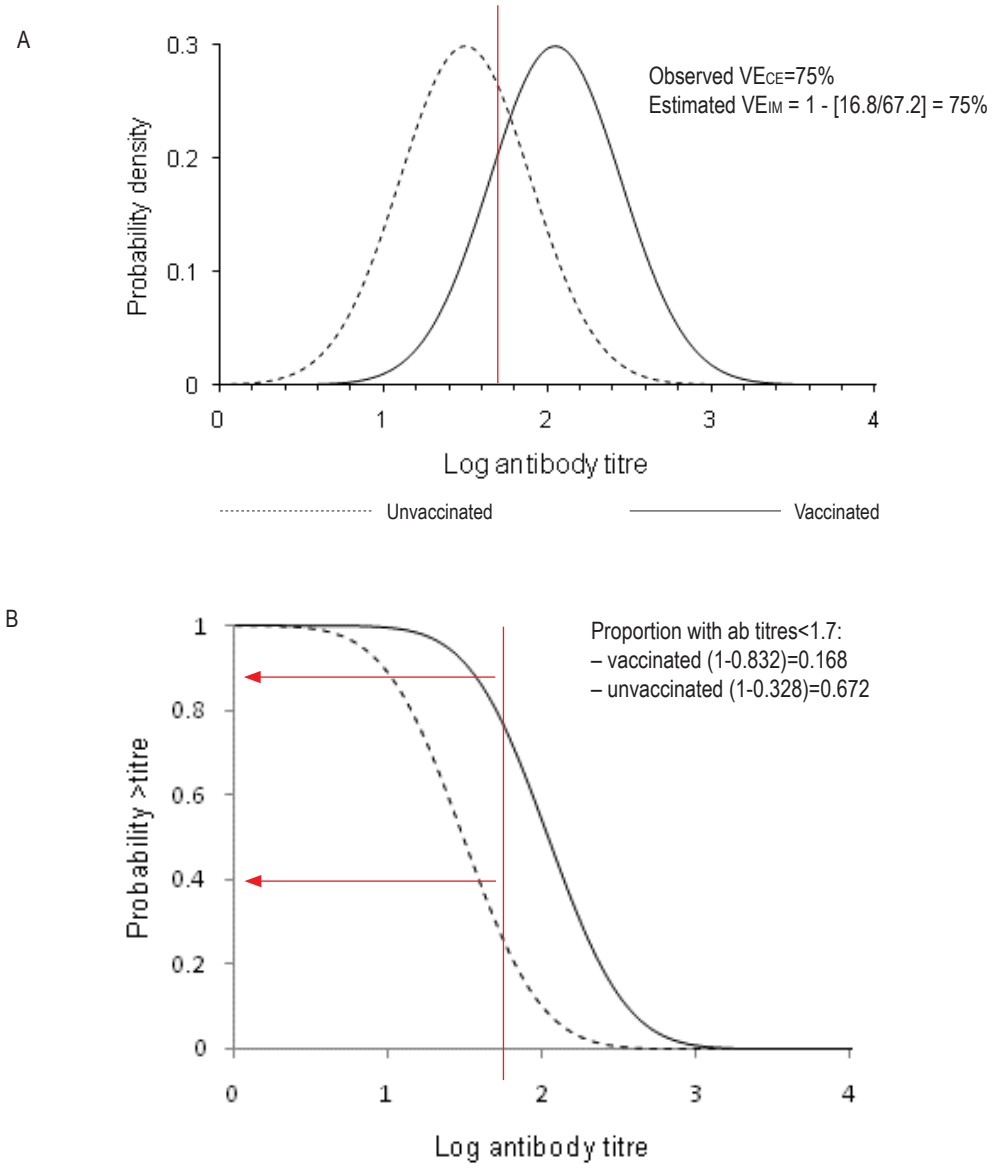
$$Equation\ 2:\ VE_{IM} = 1 - \frac{proportion\ of\ individuals\ vaccinated\ with\ immune\ marker\ titre\ below\ the\ threshold}{proportion\ of\ individuals\ unvaccinated\ with\ immune\ marker\ titre\ below\ the\ threshold}$$

VE, vaccine efficacy; IM, immune marker

The VE_{IM} can be derived by measuring immune marker titres in vaccinated and unvaccinated individuals and presenting them graphically, either as distributions of log-transformed titres for each group (as in Figure 14A) or as reverse cumulative distributions (as in Figure 14B). Such figures allow inferences from observed VE_{CE} to estimated immune marker thresholds for protection, or vice versa, from known

protective thresholds to estimated clinical VE, based upon equation 2. For example, if VE_{CE} has been estimated clinically, VE_{IM} can be estimated from the graph for each possible immune marker threshold using equation 2, to identify the threshold for which VE_{IM} equals VE_{CE}.

Figure 14. Graphic display of distributions of antibody titres in vaccinated and unvaccinated individuals, allowing inference from observed vaccine efficacy to protective threshold



A) Distributions of log antibody titres, adapted from Dunning et al. (57); VE is estimated from the graph for each possible threshold using equation 2. In this example, the estimated VE is equal to the observed VE (75%) if an antibody threshold value of 1.7 is assumed to be protective (67.2% of unvaccinated and 16.8% of vaccinated individuals have an antibody titre below this threshold).

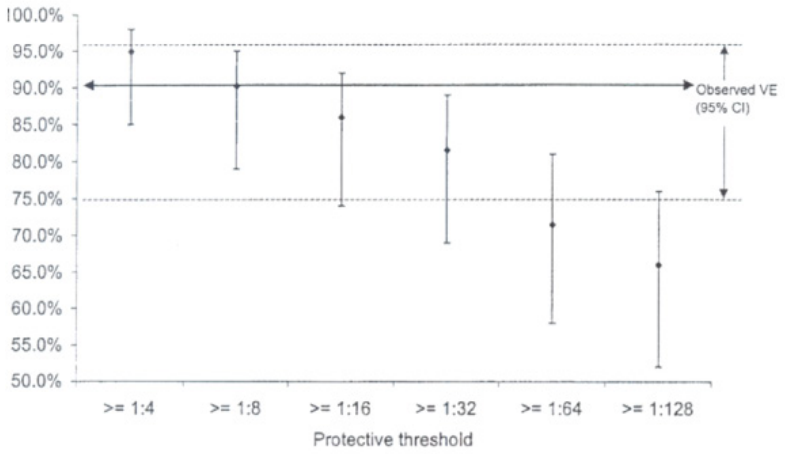
B) Reverse cumulative distribution of antibody titres in vaccinated and unvaccinated individuals, showing the same calculation.

EXAMPLES

Siber et al. (53) report the statistical methods used by a WHO working group to derive the concentration of anti-pneumococcal capsular polysaccharide antibodies to pneumococcal conjugate vaccine (PCV) that predicts protection against IPD in immunized infants. They pooled results from three RCTs of pneumococcal VE − two trials in the USA (one in Californian infants and the other in American Indian infants) using the USA schedule of 2, 4, 6, and 12 months with 7-valent pneumococcal vaccine, and one in Soweto, South Africa, using the EPI schedule of 6, 10 and 14 weeks with 9-valent pneumococcal vaccine. The South African trial data were restricted to efficacy against the seven serotypes present in the vaccine used in US trials. The protective titre was derived graphically from the reverse cumulative distribution of antibody concentrations in vaccinated and unvaccinated infants, as in Figure 14B above. A concentration of IgG anticapsular polysaccharide antibodies of ≥0.35 µg/ml measured by ELISA one month after primary immunization was recommended as the protective threshold and as the basis for licensing PCV.

Andrews et al. (58) used the threshold method to re-evaluate and validate titres of serum bactericidal antibody (SBA) as a substitute endpoint for meningococcal C conjugate vaccine-induced protection after the SBA assay was changed to use rabbit rather than human complement (as mentioned in Section 3.1.2). Post-licensure surveillance data were used to estimate observed vaccine effectiveness (VE), and this was compared with predicted VE calculated for a range of different assay cut-offs, using the step-function assumption and equation 2 above. Among toddlers, an rSBA cut-off of 8 at one month post-vaccination gave the closest estimated VE to that observed in the first year after vaccination (Figure 15).

Figure 15. Predicted vaccine efficacy based on the proportions of vaccinated and unvaccinated toddlers with rSBA titres below a range of cut-offs, measured one month after vaccination with meningococcal C conjugate vaccine (see equation 2)



Source: reprinted from Andrews et al. (58) with permission from Elsevier.

4.2.3 Continuous method

It is also possible to relate immune marker titres to disease risk and hence to VE without assuming a threshold titre associated with protection. Siber (54) describes the calculation of antibody titre-specific rates of disease by dividing the number of individuals with disease who had a specific pre-exposure titre by the number of individuals in the entire study population with that titre, and used this to infer the overall proportion protected against disease. Other researchers have fitted regression models to antibody titres in vaccinees and non-vaccinees in order to describe a statistical association between pre-exposure antibody titres and disease incidence and thus to estimate VE.

EXAMPLES

Chan et al. (43) examined a range of different regression models to describe the association between antibody titres after varicella vaccination and subsequent disease incidence in order to infer clinical protection. They showed that VE predicted by the models was similar to that observed from seven-year follow-up data from a varicella VE trial (97.4% compared to 97.0%, respectively).

As outlined in Section 4.1.2, Storsaeter et al. developed a logistic regression model to quantify the relationship between antibodies to three different pertussis antigens and vaccine-derived protection against clinical pertussis after household exposure, in a vaccine trial in Sweden. This model was subsequently validated by Kohberger et al. (46−47) using Prentice criteria and meta-analysis. The VE predicted from the model was reasonably consistent with the results from a second vaccine trial in Sweden.

Dunning (59) highlighted the inability of existing models to capture appropriately the relationship between antibody titre and clinical protection in individuals with low antibody titres, in whom the risk of disease could be highly influenced by the probability of exposure to an infectious contact, the prevalence of infection or other factors independent of the antibody titre. He proposed a new (scaled logit) model which includes a continuous relationship between levels of the immune marker and protection in which exposure is modelled explicitly. This approach has been used subsequently to model the relationship between CMI responses to influenza vaccination in children and protection against culture-confirmed clinical infection with wild-type influenza virus (60).

4.2.4 Summary

There are various ways to relate immune markers to VE. As described in this Section, some methods use antibody titres as continuous variables to predict VE, whereas other methods transform this information into a dichotomous variable – a *threshold* level of an immune marker above which subjects are assumed to be protected and below which they are not. Both approaches have their limitations. As discussed in section 4.1.1, most methods presented here stratify on the immune response, and are thus susceptible to post-randomisation bias if using data from a randomised trial. It is therefore necessary whenever possible to adjust for factors other than the vaccine that could affect the risk of infection/disease. The relationship between immune marker titres and clinical protection is further discussed in Section 6.

5. Other issues related to immunological markers for protection

5.1 Endpoint definition

It is important to specify explicitly the clinical endpoint against which the vaccine is expected to protect, since the relationship may vary depending on this outcome. Endpoints of interest include infection, illness, death, carriage, infectiousness and (for diseases such as malaria that are almost universal in certain settings) time to first endpoint or total number of disease episodes. Protection against clinical endpoints may require not just different quantities of a specific immune marker but involve different markers.

EXAMPLES

Studies of smallpox vaccine suggest that high antibody titres protect against infection, but that both antibody and T cell responses are needed to protect against severe disease (61).

For Hib, anti-PRP antibody titres of ≥0.15 mg/mL, ≥1 mg/mL and ≥5 mg/mL have been proposed as protective against short-term invasive disease, long-term invasive disease and colonization, respectively (62).

There is evidence that the measured efficacy of conventional pertussis vaccines is higher against severe than against mild disease endpoints, and continued discussion over whether these vaccines are more protective against disease than against infection (63–64).

A major issue in the evaluation of polio vaccines is that IPV is less effective than OPV in protecting against enteric infection and transmissibility, although either may be equally effective in protecting against paralytic disease (65–67).

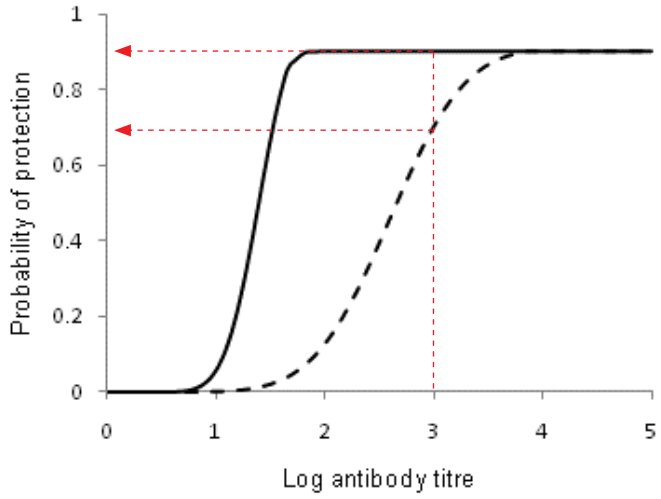
5.2 Exposure intensity/challenge dose

It may be possible to control infectious challenge doses to within a narrow range under laboratory conditions. However, this is not what occurs in human communities as there is likely to be considerable variation in exposure intensity both within and between populations. This heterogeneity of exposure is associated with factors such as whether exposure occurs within a home or other environment conducive to transmission, and individual social and hygiene behaviour. The risk of exposure to airborne or waterborne pathogens is likely to be higher in crowded and deprived settings with poor hygiene and sanitation compared to populations living in better conditions.

This has important implications insofar as protection against a higher challenge dose or more frequent exposure is likely to require higher levels of immune response (e.g. higher levels of neutralizing serum antibodies). As a consequence, the level of protection provided by a specific antibody titre in one population or setting may be higher than that in a different population or setting in which higher or multiple challenge doses are more frequent.

These factors have two implications for the relationship between protection and antibody titre, as illustrated in Figure 16. The slope or horizontal range of the curve is likely to be a function of the heterogeneity of exposure in a population, itself a function of environmental and socioeconomic heterogeneity. Its position along the horizontal axis will be a function of the average level of exposure in the population. These differences are illustrated by two curves, one (B) with a broad range or slope, indicating considerable within-population heterogeneity, in which an antibody titre of 3 is associated with 70% protection, and another (A) with a narrower slope, indicating less within-population heterogeneity, in which an antibody titre of 3 is associated with greater – in this example 90% – protection.

Figure 16. Hypothetical relationships between antibody titres and clinical protection in two populations with different intensities of exposure and different levels of heterogeneity



Population A – low exposure challenge dose, low heterogeneity

Population B – high exposure challenge dose, high heterogeneity

EXAMPLE

Taranger et al. (31) reported that children with household exposure who escaped pertussis illness had a higher titre of anti-pertussis toxin IgG antibodies post-vaccination than those who escaped illness after only extra-household exposure. This means that if the logistic regression model proposed by Storsaeter et al. (46) were used in a setting in which most transmission occurred outside the household, the VE estimate would be higher than that obtained from a population in which most transmission occurs within the household.

These within- and between-population heterogeneities need to be considered before predicting the performance of a vaccine in a population based on a substitute endpoint derived in any particular context. Ideally, immune markers are needed that can be used reliably as a substitute for clinical protection across a range of settings (the level 2 surrogate of protection described by Qin et al. (4)), or optimally in all settings. This is difficult to achieve, since many of the issues discussed in this report relate to heterogeneity between settings. The population, the pathogen strains and even the environment may be different, and these may influence the relationship between immunological markers and VE.

5.3 Host factors

Several host factors influence immune responsiveness as well as exposure doses, and hence may be expected to influence correlates of protection.

5.3.1 Age

Changes occur in the immune system over the course of the life span, exemplified by the acquisition and loss of passive immunity associated with maternal antibodies, maturation of immune competence in early childhood (which is for example relevant to the ability to mount a response to polysaccharide vaccines), repeated exposure to a wide variety of (sometimes cross-reactive) antigens throughout life, and progressive immunosenescence in old age. It is likely that for some pathogens, the components of the immune response important for protection (and thus predictive of VE) change quantitatively and qualitatively with age.

EXAMPLE

While it is commonly assumed that the efficacy of inactivated influenza vaccines is predicted by serum HI antibody titres, Gravenstein et al. (68) found that in a cohort of 408 nursing home residents aged ≥65 years, 22 of those with high vaccine-induced HI titres (>1:640) still developed laboratory-confirmed influenza illness. A study of the efficacy of an inactivated influenza vaccine by McElhaney et al. (69) in the same cohort suggested that the CMI response might be a better predictor of protection against laboratory diagnosed influenza compared to humoral immunity. This evidence was based only on examination of the VE among older individuals (note - their immune response was not compared directly to younger individuals to assess whether CMI is also a better predictor of protection in a younger age group).

5.3.2 Socioeconomic status

Since people from deprived socioeconomic backgrounds are more likely to live in crowded settings, socioeconomic status (SES) is associated with parameters that can affect the host response to the vaccine, such as nutritional status, exposure dose and frequency of exposure. As a result, the predictive value of a substitute endpoint may differ among persons from different settings or SES (as illustrated in Figure 16).

EXAMPLE

A concentration of IgG anticapsular polysaccharide antibodies of ≥0.35 µg/ml one month after primary immunization was used for the licensing of PCV, based on pooled data from three trials (53, 70). In the individual trials, the antibody titre associated with protection was higher among infants more likely to be living in crowded and deprived settings, i.e. American Indian infants (≥0.99 µg/mL) and those enrolled in the trial carried out in Soweto, South Africa (≥0.68 µg/mL) compared to those living in northern California, USA (≥0.20 µg/mL). This heterogeneity in the antibody threshold is likely to be due to higher exposure dose among infants from more deprived settings. The recommended threshold (≥0.35 µg/mL) to assess pneumococcal VE could thus overestimate a vaccine's efficacy in lower SES settings and underestimate efficacy in higher SES settings.

5.3.3 Environmental factors

There are several instances in which vaccines perform very differently depending on the environment. This may be attributable to socioeconomic factors, but may also relate to climatic or other features of the environment.

EXAMPLES

There is much evidence that variations in BCG protection aganst tuberculosis are attributable at least in part to regional differences in exposure to environmental mycobacteria that share antigens with BCG or the tubercle bacilli, and either mask or block the action of the vaccine (71).

The Gambian trial of pneumococcal conjugate vaccines noted that GMC antibody concentrations were significantly higher among those vaccinated in the rainy season (15).

5.4 Antigen factors

Most of the statistical methods used to predict VE based on substitute endpoints assume that the relationship between the immunological marker and clinical protection is similar among the vaccinated and unvaccinated groups. This assumption ignores the fact that any immunity observed in unvaccinated individuals may be derived from natural exposure to antigens that are not in the vaccine (i.e. from exposure to the pathogen itself, to related organisms or to other antigens in the environment that share epitopes with the infectious agent in question), and can thus be qualitatively and/or quantitatively different from that observed among those vaccinated.

EXAMPLE

Live measles vaccines are attenuated strains of wild measles virus. Itoh et al. (72) discuss qualitative and quantitative differences between vaccine-induced and naturally acquired antibody response against measles in Japan. They showed that among children aged less than 10 years, those who had been vaccinated had significantly lower titres of measles neutralizing antibody than those who had experienced natural infection (mean titre ±SD: 4.8 ±1.5 versus 8.0 ±1.1, $p<0.0001$). They also found differences in the relationship between neutralizing and HI antibody activities: among those with similar levels of HI activity, vaccinated children had significantly lower neutralizing antibody titres than naturally infected children, indicating that there were qualitative differences between anti-measles antibodies produced after natural infection and after vaccination. It is unclear whether any such differences might be attributable to either attenuation of the vaccine virus or to the "unnatural" (intramuscular) presentation of vaccine-derived measles virus compared to wild virus.

Similarly, the immune response induced against an infectious agent may vary depending on the composition of the vaccine. For example, subunit vaccines comprise only part of a pathogen and will therefore elicit a different immune repertoire to that produced following natural exposure or vaccination with whole live or killed organisms.

EXAMPLES

Jelonek et al. (73) compared "natural" immunity from Hib exposure to that derived from four different vaccine formulations, a simple polysaccharide vaccine and three conjugate formulations that differed in composition and structure. They found that Hib vaccines yielded slightly different qualitative and quantitative antibody repertoires, and that each repertoire was different from the response elicited by natural infection. Findings from a study by Schlesinger et al. (74) suggested that antibodies induced by different anti-Hib conjugate vaccines had differing avidity; for instance, antibodies elicited by the HbOC formulation showed higher avidities and better bactericidal activity than the PRP-OMPC formulation.

Antibody responses to pertussis vaccines depend on the antigens present in the particular vaccine (whole cell versus acellular, number of components), and their dosage (75). For instance, the antibody repertoire elicited by the 2-component (PT, FHA) and the 5-component vaccines (PT, FHA, PTN, and two types of FIM) are very different.

5.5 Immunological factors

5.5.1 Type of antibody

Most regulatory bodies prefer, whenever possible, to measure functional antibodies instead of total antibody level (which might include inactive antibodies). This provides biological plausibility for any association found between immunological markers and clinical protection, and increases confidence in the VE measures estimated using such markers as substitute endpoints. Examples of functional antibody assays include neutralizing antibodies (e.g. PRN for measles (5)), and bactericidal and opsonophagocytic antibodies (OPA) for polysaccharide vaccines (e.g. pneumococcal or Hib vaccines) (76–77). Thus, when assessing potential immune markers as substitute endpoints for VE, both qualitative and quantitative aspects of the immune response need to be considered. These include the avidity and bactericidal activity of an antibody and the association of titres of that antibody with the level of clinical protection.

EXAMPLES

Saaka et al. (15) reported findings from a subgroup of children enrolled in an efficacy trial of 9-valent pneumococcal conjugate vaccine (which included serotype 1) in the Gambia. Most vaccinated children achieved a serotype 1-specific antibody level of >0.35 µg/mL (the WHO recommended threshold), and 98% had a high ELISA geometric mean concentration (GMC) of >5 µg/mL. Surprisingly, the trial results were compatible with no protection induced by the vaccine against serotype 1 IPD (serotype 1-specific VE = -98% (95% CI -2090% - 72%). Although the number of events was small (4 cases of serotype 1 IPD in the vaccinated versus 2 in the placebo), the authors conjectured that antibodies specific to this pneumococcal serotype could have had lower functional activity (e.g. low opsonophagocytic activity).

Schuerman et al. (78) showed that, although there was a correlation between serotype-specific OPA GMCs after vaccination with pneumococcal conjugate vaccine and protection against acute otitis media in young children, there was a lack of correlation between circulating IgG levels (measured by ELISA) for individual serotypes and clinical protection.

5.5.2 Kinetics of the immune response

The measurement of any immunological marker is a snapshot, which needs to be interpreted in the light of the kinetics of the marker. Among several examples, Olin et al. and Kohberger et al. (47, 79, respectively) have both commented that the kinetics of antibody to pertussis vaccination may explain the lack of correlation between pertussis antibodies and clinical protection in some studies, particularly those that measured antibody responses very early after vaccination rather than at the time of later exposure. For hepatitis B vaccine, it has been shown that the anti-HB antibody titre rises to a peak after vaccination and then decreases progressively (25, 80–81). Analyses suggest that the post-vaccination peak titre could be the optimal antibody measure as a marker for protection against persistent infection.

Unlike the hepatitis B example, antibody titres against some viruses appear to remain stable for a long time, even in the absence of boosting. For example, Amanna et al. (82) estimated, on the basis of data from a cohort of 45 adults, that the lower boundary of the 95% CI for the half-lives of antibodies against rubella, measles, mumps, and hepatitis B virus were 114, 104, 90 and 63 years, respectively.

Related to this is the issue of memory and the anamnestic response, whereby individuals can mount protective responses rapidly from a low background antibody titre level. Individuals with such vaccine-induced memory capability can in some circumstances be well protected despite a low antibody titre post vaccination or at the time of exposure.

Passive immunization and mother-to-child passive immunity transfer were discussed in Section 3 as possible means to identify potential candidate immunological markers of vaccine-induced protection. These study designs implicitly ignore the redundancy of the immune system and any potential cooperation between adaptive CMI and humoral immunity. This is further complicated by the fact that the kinetics of different arms of the immune system are likely to differ.

5.6 Measurement error and noise

Statistical methods assume that endpoints are measured accurately and do not account for measurement error. Non-systematic measurement error (e.g. arising from lack of standardization, laboratory variability or high intra-individual variability of the marker) can attenuate an association between a substitute endpoint and protection, and between the substitute endpoint and the vaccine. A consequence of this, for example in a Prentice-like validation, will be that adjustment for the substitute endpoint may fail to remove all the effect of the vaccine on the clinical endpoint. Thus an important component of the search for immune correlates is assay validation studies and selection of assays with the lowest 'noise'.

Among the tools used to investigate the reliability of measurements and the contribution of intra- and inter-individual variations is the intra-class correlation coefficient (ICC). In theory, the inter-individual variation should be greater than the intra-individual variation in order for a substitute endpoint to be useful. Intra-individual variation may be linked to factors such as the kinetics of the immune marker (short or long-term fluctuation over time) and the reliability of the assay. Various approaches are used to reduce intra-individual variation, including taking repeated measurements at different times and/or repeated samples at approximately the same time, using identical storage methods for samples, and standardized assays. Most of the inter-individual variations observed in the immune response are expected to be explained by the effect of the vaccine, but residual variation may arise due to differences among individuals in their nutritional status, genetic traits, intensity and frequency of pathogen exposure, etc. The residual variation can be minimized by randomization in a trial or by adjustment and other methods for control of confounding in observational studies (83).

Assays used to measure potential immunological markers of vaccine-induced protection may give different results depending on the laboratory in which the samples are processed. The need for valid and reliable assays is thus emphasized by regulatory agencies.

EXAMPLE

WHO recommends measurement of the opsonophagocytic activity of vaccine-induced antibodies as a criterion for pneumococcal conjugate vaccine licensure. It was acknowledged at a recent WHO workshop (84) that the opsonophagocytic activity assays are not yet sufficiently standardized.

6. Putting it together – the relationship between immunological markers and vaccine performance in the field

6.1 Interpretation of vaccine-induced protection: 'all-or-none' versus 'partial' models

When a subject gets complicated, it is sometimes useful to go back to the initial questions. What is meant by a vaccine conferring 80% protection against a specific clinical endpoint, and how does this relate to levels of immune markers that may be used as substitute endpoints? Smith et al. (85) proposed two models of vaccine action, termed all-or-none (according to which an efficacy of 80% implies that 80% of vaccine recipients are totally protected, and the remaining 20% not at all protected) or partial (whereby all recipients of the vaccine have their incidence rate reduced by 80% compared to non-vaccinees). The initial paper emphasized the implications of these two models for the measurement of VE using observational studies. This distinction has since been discussed by several authors, some of whom have used the term "leaky" to describe the partial model of Smith et al. (86–88).

This perspective provides an opportunity to tie together several of the themes introduced above, by relating the presumed mode of action of vaccines to immune markers, clinical endpoints and VE. It is important to note that the models were initially proposed from an epidemiological perspective – reflecting what is observed in terms of risks of outcome among vaccinees and non-vaccinees over time in populations. Interpretation of these models brings together several issues, including: (a) what the vaccine does to the immune response, (b) the implications of various immune responses for protection, and (c) the implication of variations in challenge dose in the community.

6.1.1 All-or-none model of action

This model assumes that a certain proportion (VE %) of vaccine recipients reach a threshold level of (for example) antibody titre and are fully protected against the disease, and that all the remaining (100–VE%) are not protected at all – they remain as susceptible as those who had not been vaccinated. There are at least two ways to conceive of this circumstance, described in A and B below.

All-or-none model A

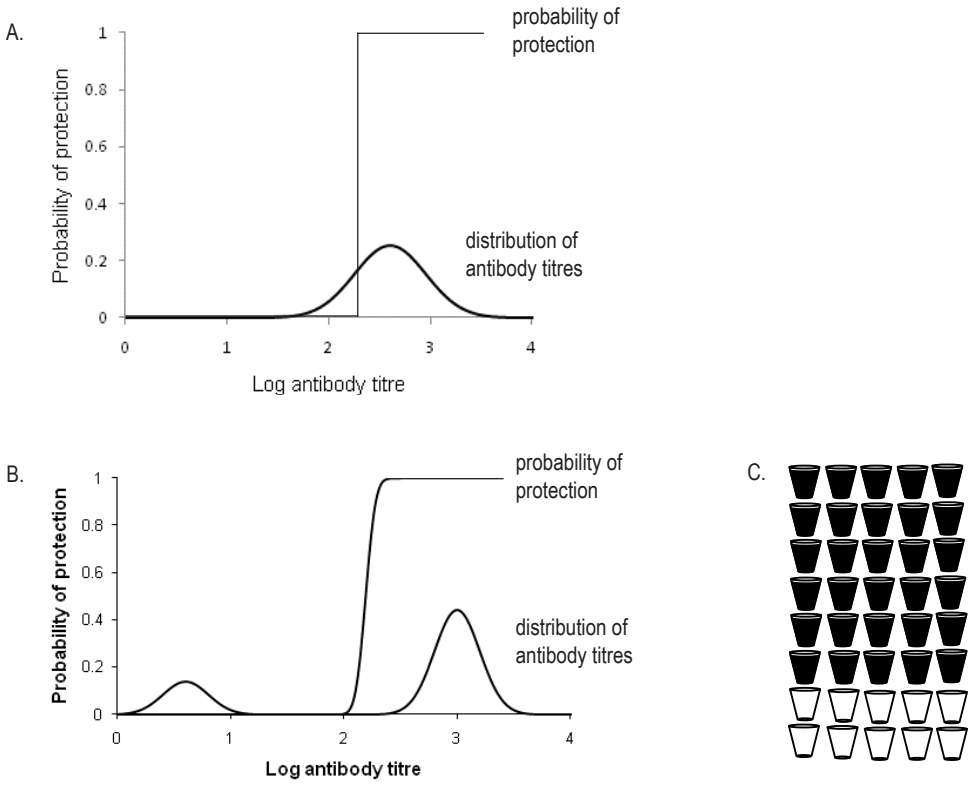
The most straightforward interpretation of this model is to consider the relationship between the antibody level and protection as a simple step function, with 0% protection up to a threshold antibody titre and 100% protection thereafter, as discussed in Section 4.2.2 and shown in Figure 17A. The vaccine is assumed to raise a proportion (VE %) of vaccinees above this threshold.

All-or-none model B

An alternative interpretation of the all-or-none model is to suppose that the relationship between antibody titre and protection is step (or sigmoid) in shape and that individuals with high titres are completely protected, but that the vaccine's effect on recipients is distinctly bimodal, such that the immune response of a proportion (VE %) of vaccinees is lifted to the higher level associated with complete protection, and the vaccine has no effect on the remaining 100–VE% of vaccinees. This is illustrated in Figure 17B.

The important point with either interpretation is that a certain proportion (VE %) of vaccinees are fully protected, and the remaining 100–VE% are not protected at all by the vaccine. This is illustrated in Figure 17C, in which the goblets represent vaccinated individuals, and the liquid in each goblet represents the level of protection. The array indicates that 75% of the vaccinated individuals are fully protected and 25% of them are not protected at all. Thus, VE is 75%.

Figure 17. Relationship between log antibody titre and clinical protection if challenged in an all-or-none model



Distribution of log antibody titres among vaccinees: unimodal (A, bottom line), bimodal (B, bottom line).

In the first interpretation, the step threshold may be considered a simple correlate – or surrogate – of protection. In the second, the correlate could in theory be any titre which separates the fully-protected vaccinees from the remaining (unprotected) vaccine recipients. These two antibody titre thresholds may be close to what Plotkin (89-90) calls "absolute correlates of protection". He gives examples, including vaccines against diphtheria, tetanus, measles, rubella, hepatitis A and Lyme disease.

It may be that a circumstance such as illustrated in Figure 17A is unlikely for many pathogens. A unimodal distribution of antibody titres among vaccinees and a simple step threshold between no and full protection seems biologically less plausible than the sort of circumstance illustrated in Figure 17B.

If the all-or-none model were to hold, the antibody titre threshold could be considered as a quantitative substitute for protection and used for licensure of a vaccine. Once the threshold has been determined, the VE measured using this substitute endpoint could be estimated by comparing the proportion of individuals below this threshold in the vaccinated group to that of the placebo group (as in Section 4.2.2, equation 2). Protective antibody titres for several vaccines, including pneumococcal and meningococcal C conjugate vaccines, have been derived using methods that in effect assume that the vaccine mechanism corresponds to an all-or-none model.

This perspective ignores whether or not the immune marker is actually on a causal pathway. If an all-or-none mechanism is accepted, with perfect correlation between an immune marker and observed clinical protection, the association should allow strong inferences for licensure purposes – regardless of whether the immune marker is itself involved in protection.

6.1.2 Partial ('leaky') models

According to this model, the risk of infection/disease in all vaccinees is reduced (by VE %) compared to non-vaccinees, none of the vaccinees being fully protected. The assumption that no vaccinee is totally or permanently protected implies one or both of the following:

i) No amount (titre) of the immune marker is totally protective or, if it is, no individual can maintain that titre for a long period (because of waning or transient immunosuppression);

ii) The degree of protection is a function of the level of the immune marker – the simplest explanation being that protection is a function of both the level of the immune marker and the challenge dose.

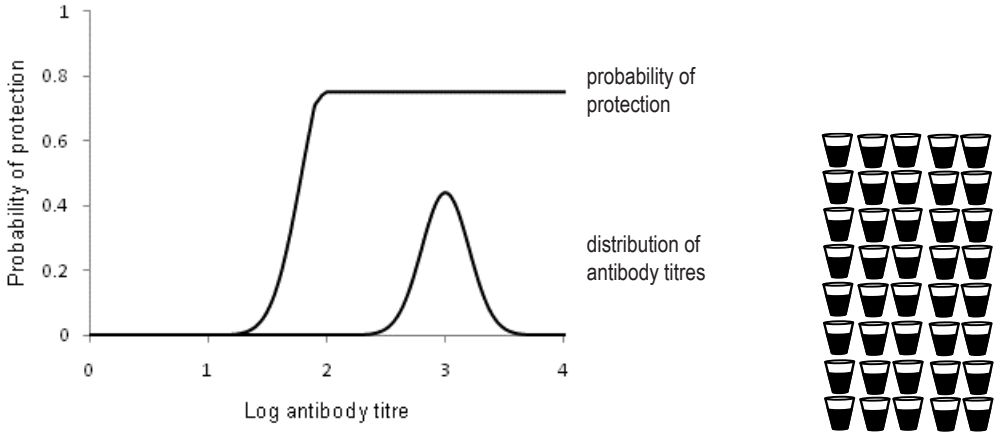
Two interpretations follow:

Partial/leaky model A

According to this interpretation, protection is a step function such that those above some immune marker threshold have their risk reduced by VE % compared to those below the threshold – and the vaccine succeeds in raising all vaccinees above this threshold. As illustrated in Figure 18, partial (75%) reduction of disease risk is attained at antibody titres above a threshold of approximately 2.0. The goblets indicate a circumstance in which all vaccinated individuals are 75% protected. In this simplest description, the threshold antibody level is sufficient to protect against VE % of challenge doses in

the population. In theory, if follow-up were to continue a very long time, all vaccinees will ultimately be exposed to a higher challenge against which the above-threshold antibody cannot protect, and thus everyone will ultimately contract the outcome / illness.

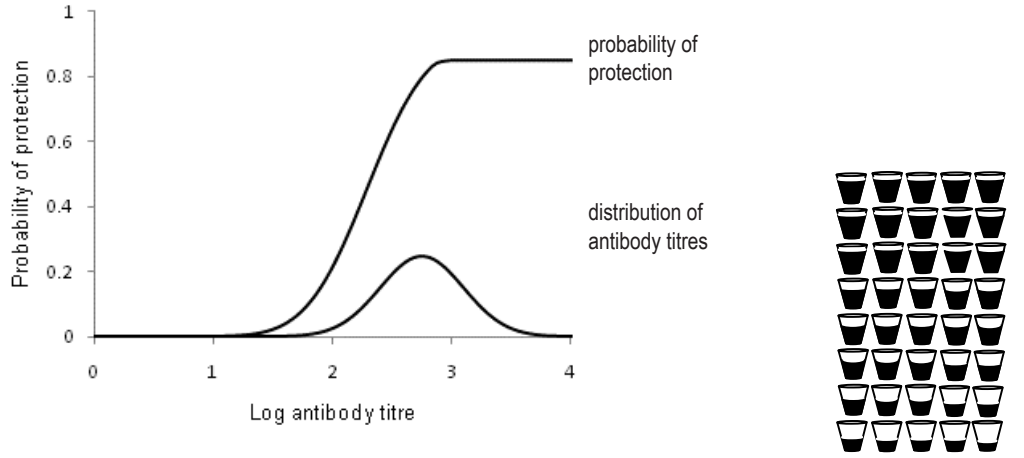
Figure 18. Relationship between antibody titre and the probability of protection in a partial model



Partial/leaky model B

According to this interpretation, the level of protection is a function of the antibody level over a range of antibody titres induced by the vaccine. In this situation, overall VE may be interpreted as an average reduction in risk of disease among vaccinated compared to unvaccinated individuals. This is illustrated in Figure 19, which corresponds to Dunning's scaled logit model (59) mentioned in Section 4.2.3; it is possible to use the knowledge of the probability of disease to different levels of the immune response to estimate VE correctly. As with partial/leaky model A, no one has 100% protection and so in theory all will ultimately succumb to the clinical outcome if followed for long enough.

Figure 19. Relationship between log antibody titre and clinical protection if challenged and the distribution of log antibody titres among v accinees in an alternative partial model



The goblets represent a vaccinated population with increasing levels of protection up to 85% protection.

Partial protection models have interesting properties. According to the model B interpretation, those with the lowest levels of antibody induced by the vaccine, and hence with the least vaccine-induced protection, should contract the infection more rapidly than those with the higher levels of antibody and protection, and hence one expects observed protection to increase over time.

6.1.3 Summary

Although the all-or-none and partial/leaky models provide useful perspectives, it is likely that neither describes well what really happens, and that a variety of hybrid mechanisms apply in the real world. For example, there may be circumstances in which some individual vaccinees are not at all affected by the vaccine (their goblets remain empty), but others respond in various ways giving them different levels of protection. This might be viewed in terms introduced in Section 1.1.2: some achieve complete protection (the full goblet – protection against any challenge), some achieve incomplete protection (partially full goblets, implying protection against low or moderate, but not against high challenge doses); and some achieve no protection at all (the empty goblet, effectively no different from a non-vaccinee).

This perspective may be most useful in clarifying the importance of heterogeneity – both in terms of immune response among vaccinees (represented by the frequency distributions of titres in Figures 12, 16, 17, 18, 19) and in terms of protection (represented by the various sigmoid curves in Figures 12, 13, 16, 18, 19). Heterogeneity of immune response will be largely a function of the vaccine product, the genetic background, and prior immune experience of the vaccinee. Heterogeneity of protection will be determined largely by epidemiological factors influencing exposure intensity or environmental factors – illustrated in this document by pneumococcus and pertussis, for which intensity of exposure appears to be key, and tuberculosis, for which environment is also key.

It is implicitly assumed in many conventional interpretations of protective immunity and vaccine action that heterogeneity is not important. Fortunately, this may be the case for some vaccines and infections, e.g. measles and others with relatively clean correlates of protection, but for others this may explain much of the difficulty in vaccine evaluation (e.g. for tuberculosis or pertussis). Such heterogeneity complicates the issue of predicting protection as a function of immunological measures.

7. Conclusions

The use of immunological markers as substitute endpoints for vaccine evaluation is important, but complicated. It is not straightforward to identify such markers or to ensure that the estimated VE derived from their use predicts accurately the vaccine effectiveness that would have been observed if the clinical endpoints were recorded. Differences between infectious agents, vaccines, immune responses and population contexts provide many opportunities for heterogeneities and complex relationships.

It is intuitively reasonable that a vaccine's effectiveness in protecting against an infection or disease outcome will be a function of several things: the prior immunological status of the vaccine recipient; the immune response to the vaccine (described in terms of the proportions of individuals who respond in a particular way to any given degree); the implications of different levels and combinations of immune response for protection against various exposures; and the actual patterns and levels of exposure in the vaccinated community. These several influences are brought together in considering the various models of vaccine action, as described in Section 6.

For regulatory purposes, it may not be necessary to contemplate all the factors that can influence effectiveness, but to concentrate on two: the effect of the vaccine in terms of induced immune response, and the implications of these responses for clinical protection under some standard circumstances. Despite the methodological challenges outlined in this paper, experience is accumulating with reference to many infections and vaccines, which is considered sufficient to allow licensure on the basis of phase II immunogenicity studies and prior knowledge on effectiveness of related vaccines.

Epidemiologists involved in vaccine evaluation also need to be aware of the broader subtleties of vaccine responses and effects (issues of indirect protection and herd immunity, e.g., are not discussed in this paper). Smith et al. pointed out that time trends in observed effectiveness (apparent efficacy) of vaccines are influenced by whether an all-or-none or partial immunity model is appropriate, and these, in turn, determine what incidence statistic or control group should be employed in cohort or case–control studies to evaluate efficacy, respectively (85). Such subtleties have rarely been applied in practice to date, but they hold the opportunity for careful epidemiological studies to provide insights into the mechanism of action of vaccines.

Much work remains to be done. Indeed, this is a subject ripe for serious collaboration between immunologists, statisticians, epidemiologists and regulatory vaccinologists.

Annex 1: Correlate and surrogate terminology

The terms *correlate* and *surrogate* of protection are used widely but inconsistently in the literature, including by regulatory agencies and prominent authorities. Examples from regulatory agencies include the following.

US Food and Drug Administration (FDA)

Surrogate endpoint: "Laboratory or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful endpoint that is the direct measure of how a patient feels, functions or survives and that is expected to predict the effect of the therapy" (57 FR 13234-13235 4/15/92) (91).

Correlate of protection: "Generally, a laboratory parameter that has been shown to be associated with protection from clinical disease" (Tiernan, 92).

Accelerated approval of vaccines by the FDA can be granted if well-controlled trials have shown that a surrogate endpoint is considered "reasonably likely" to predict clinical benefit, subject to the requirement that the applicant studies the vaccine further to demonstrate clinical benefit (Norman Baylor, Global Vaccine Research Forum, Bamako, Mali, December 2009). This indicates flexibility in the definition of a surrogate endpoint so that the FDA can use expert judgement in specific circumstances.

International Conference on Harmonization (ICH): European Union, Japan and USA tripartite guidelines

Validated surrogate endpoint: "an endpoint which allows prediction of a clinically important outcome but in itself does not measure a clinical benefit. When appropriate, surrogate outcomes may be used as primary endpoints." The strength of evidence for a surrogate includes consideration of: "(i) the biological plausibility of the relationship, (ii) the demonstration in epidemiological studies of the prognostic value of the surrogate for the clinical outcome and (iii) evidence from clinical trials that treatment effects on the surrogate correspond to effects on the clinical outcome" (ICH, 93–94).

European Agency for the Evaluation of Medicinal Products (EMEA)

Serological surrogate: "a predefined antibody concentration correlating with clinical protection".

Immunological correlate of protection: "...e.g. specific antibody titre correlating with protection..." (95).

The definitions described in the above three examples have been adopted or modified by other agencies. For example, the EMEA definition of a serological surrogate was used in the WHO guidelines on clinical evaluation of vaccines (96).

The inconsistency in these definitions is accentuated by differences in the choice of the factor for which substitutes are sought. This is illustrated by contrasting the following definitions proposed by Plotkin (89-90) and by Qin et al. (4) for use in vaccine research.

Comparison of definitions of correlates and surrogates of protection

	Plotkin (2008)	**Qin et al. (2007)**
Correlate of protection / risk	Immune response closely related to protection / that provides protection	Immune response whose presence is associated with low risk of disease/ infection
Surrogate of protection	Immune response that is not in itself protective, but which substitutes for the true correlate	Correlate that predicts accurately the level of VE, i.e. for which it can be shown that it is responsible for protection

Plotkin[2] (89-90) considers a substitute for the *protective immune response*. He calls this substitute a *surrogate of protection*, defined as "a quantified specific immune response to a vaccine that is not in itself protective but that substitutes for the true (perhaps unknown) correlate". He calls the protective immune response itself a *correlate of protection*, defined as "a specific immune response to a vaccine that is closely related to protection against infection, disease, or other defined end point".

In contrast, Qin et al.[3] (4) focus on substitutes for *clinical protection*; they consider substitute endpoints that are ideally *surrogates of protection*. They define a *correlate of risk* as "an immunological measurement that correlates with the rate or level of a study end point used to measure VE in a defined population". On the other hand, a *surrogate of protection* is "a correlate of risk that reliably predicts a vaccine's level of protective efficacy on the basis of contrasts in the vaccinated and unvaccinated groups' immunological measurements".

It appears that these important authors differ in their perspectives, and that Plotkin's use of "correlate" approximates Qin's use of "surrogate". Their examples illustrate these different perspectives. For instance: Plotkin calls a PRN antibody titre of ≥120 mIU/mL a *correlate of protection* against measles (PRN antibodies are directly involved in the neutralization of the virus and therefore have a direct causal role in protection). On the other hand, he describes serum IgA against rotavirus as a *surrogate* for mucosal IgA, which he in turn calls a correlate because mucosal IgA is responsible for protection against colonization of the gut by rotavirus, not serum IgA. In contrast, Qin et al. note that serum antibodies to capsular polysaccharide of *H. influenzae* type b vaccine are correlated statistically to the *risk of* meningitis (and thus provide a good *correlate of risk*), but that the relationship between meningitis incidence and the antibody concentration is different in vaccinees and non-vaccinees. Specifically, antibody concentrations of ≥0.15 µg/mL predict low incidence in non-vaccinees whereas concentrations of ≥1 µg/mL are needed to predict equally low incidence in vaccinees, thus invalidating this *correlate of risk as a surrogate of protection*.

[2] Plotkin uses the term "correlate of protection";
[3] Qin et al. use the term "correlate of risk"

Other statistical definitions exist. For example, Prentice defined a *surrogate endpoint* as "a response variable for which a test for the null hypothesis of no relationship to the treatment groups under comparison is also a valid test of the corresponding null hypothesis based on the true endpoint" (3). Qin et al. define criteria for a *principal surrogate of protection* using a framework of causal inference, namely: "(i) groups of subjects with no or the lowest vaccine effect on the immune response have no vaccine efficacy and (ii) groups of subjects with a sufficiently large vaccine effect on the immune response have positive vaccine efficacy" (4).

Some authors have used the terms *individual-* and *population-based correlates* of protection with reference to those derived from (i) follow-up of entire vaccinated populations with known immune marker titres (called "individual-based") or (ii) derived from frequency distributions of samples of vaccinated and unvaccinated populations (called "population-based") (54).

To sum up, the definitions used to describe immune markers as substitute endpoints to evaluate vaccine efficacy are not universally agreed. There are differences in the usage of the terms *correlate* and *surrogate* of protection. In this report, the term *correlate* is favoured to describe markers that are statistically associated with clinical protection, but are not necessarily on the causal pathway leading to protection; and the term *surrogate* to refer to markers that lie on the causal pathway leading to protection. This is thus closer to Qin et al. (4) than to Plotkin (89-90) in terminological preference.

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