Immune Correlates of Protection: Recent Developments and Future Challenges

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Abstract

Immune correlates of protection are immunological assays which have been shown to be associated with protection from disease, and more particularly threshold values of assays which differentiate individuals susceptible to disease from those protected. They are of considerable interest in vaccine research. Important advances in statistical methods have been made in the last 10 years: a framework for evaluating candidate assays has been postulated, causal inference methods proposed and implemented, consistent terminology suggested and methods for finding thresholds and quantifying protection investigated. Some earlier methods have been further developed. The presentation will attempt to summarize the work done, introduce some new work, and frame the questions which might need to be addressed for an immune correlate of protection to serve in a regulatory context as a surrogate endpoint for clinical disease in a vaccine efficacy trial.

Key Words: vaccine, immunological assay, immune correlate of protection, vaccine efficacy.

1. Introduction

Immune correlates of protection are immunological assays which have been shown to be associated with protection from disease, and more particularly threshold values of assays which differentiate individuals susceptible to disease from those protected. They are of considerable interest in vaccine research. From data on assay values and subsequent disease occurrence collected in a successful vaccine efficacy trial of a novel vaccine, an assay reliably predicting protection and a threshold indicative of protection may be found, which may then be used to assess the effects of co-administration with other vaccines, in the development of combination vaccines, for modelling the effectiveness of proposed vaccination programs, and potentially in the licensure of next-generation vaccines. When an efficacy trial is not successful, such data can help shed light on the mechanisms of action of the vaccine and the pathogen.

Two questions have been the subject of statistical enquiry: What statistical properties of an immunological assay demonstrate that it reliably predicts protection? and How shall a threshold assay value differentiating susceptible from protected individuals or other quantification of protection be found?

We here attempt to present in outline the statistical methods which have been developed to address each of these questions, terminology that has been proposed to describe assays having particular characteristics, and to identify some of the challenges remaining to be addressed. However, this outline is able to provide only the briefest introduction to the

topics covered, and the reader is strongly encouraged to consult the references given, the citations in those references, work citing those references and other research for a more detailed and complete elucidation of each topic.

2. Which assay?

The task of selecting an assay which reliably predicts protection has received extensive consideration in the context of the search for a preventative HIV vaccine. In this setting, the mechanisms of HIV-1 transmission are poorly understood and hence the intended mechanism of action of the vaccine cannot be known definitively in advance of an efficacy trial. Consider as a motivating example the RV144 trial, a randomized trial conducted in Thailand of a recombinant canarypox vector vaccine plus two booster injections of a recombinant glycoprotein 120 subunit vaccine; a short list of six antibody or cellular assays which met pre-specified criteria were chosen for primary analysis to determine the roles of T-cell, IgG antibody, and IgA antibody responses in the modulation of infection risk [1]:

- the binding of plasma IgA antibodies to HIV-1 envelope proteins (Env)
- the avidity of IgG antibodies for Env
- antibody-dependent cellular cytotoxicity,
- HIV-1 neutralizing antibodies,
- the binding of IgG antibodies to variable regions 1 and 2 (V1V2) of the gp120 Env,
- the level of Env-specific CD4+ T cells.

Finding the assay best associated with protection could greatly assist assessment of alternative vaccines, and potentially guide the development of vaccines by shedding light on mechanisms of action.

The Qin and Gilbert framework proposed the elements necessary for an assay to reliably predict protection from disease [2,3]. It posits first that a correlation must be shown between the rate of the clinical endpoint and pre-exposure assay values. Appropriately applied logistic regression could be used to demonstrate this, and the assay may then be termed a ‘correlate of risk’. To be a ‘surrogate of protection’, vaccination should both increase assay values and reduce the rate of disease among vaccinees, and at least one of two further conditions should be met. Either the probability of disease may be shown to meet the Prentice criterion, i.e. that for a given assay value the probability of disease is independent of the treatment group, or equivalently that the relationship between assay value and disease is the same for vaccinees and placebo recipients [4]. (A similar method, using a test statistic with the same numerator but a different denominator, is the Proportion of treatment effect explained [5]; both methods require variability in assay values of placebo recipients to yield meaningful results). Alternatively, a potential outcomes approach, based on the principal surrogate framework of causal inference [6] may be used to show that vaccine efficacy is low among those with low post-vaccination assay values and increases with increasing assay value; (lack of variability in the assay values of placebo recipients is not an impediment for this method). Surrogates of protection may be used to predict vaccine efficacy in settings and populations similar to those in which they were derived; in addition, if the conditions are shown to hold in a variety of populations and settings, the assay might be used to reliably predict vaccine efficacy more generally without the need for clinical endpoint trials.

Methods based on causal inference would appear to be particularly relevant to the question of Which assay? since subjects cannot be randomized to assay values, and hence standard methods inferring causality from randomization cannot be applied. A correlation between assay values and rate of disease may merely result from subjects' more- and less-robust immune systems.

A development of the causal inference approach is the vaccine efficacy curve method [7,8], showing how vaccine efficacy varies with post-vaccination assay values of vaccinees, and is illustrated for two hypothetical assays in Figure 1.

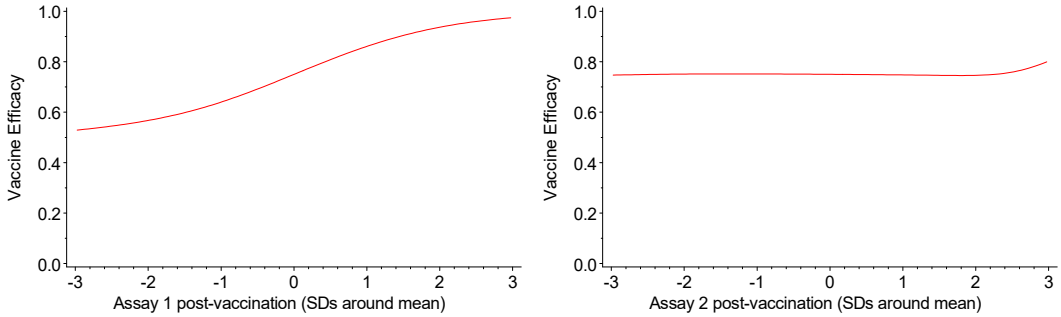

Figure 1. Illustration of two hypothetical vaccine efficacy curves.

A steeper vaccine efficacy curve indicates a stronger association between assay and vaccine efficacy, suggesting the assay better reflects the effects of vaccination.

A vaccine efficacy curve is constructed from the estimated probability of disease among vaccinees and the estimated probability of disease among placebo recipients, both as functions of post-vaccination assay value, as shown in Figure 2.

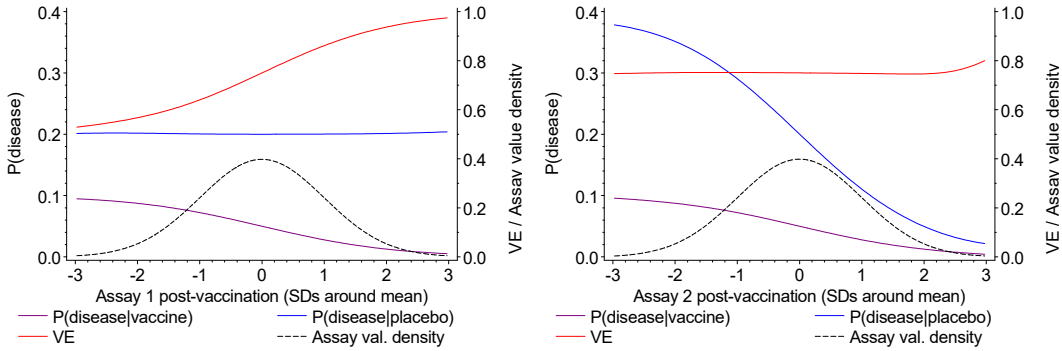

Figure 2. Illustration of two hypothetical vaccine efficacy curves, showing their components – the estimated probability of disease among vaccinees as a function of post-vaccination assay value, and the estimated probability of disease among placebo recipients as a function of post-vaccination assay value.

A flatter VE curve reflects subjects' more- and less-robust immune systems, those with more robust systems responding to vaccination with higher assay values and less disease, but also experiencing less disease when not vaccinated.

The post-vaccination assay values of placebo recipients are not observable, and the assay values they would have had if they had been vaccinated must be imputed. Follmann

proposed two methods for doing this, close-out placebo vaccination and baseline irrelevant vaccination [9]. A variation of the latter is the baseline immunogenicity predictor method of Qin and Gilbert [10], which has been applied to find a correlate of protection for herpes zoster vaccine [11], though the question of whether the conclusion is unique or whether it depends on the predictor used has been raised [12]. Whereas Follmann and Qin and Gilbert used an estimated likelihood approach, multiple imputation has also been proposed [13].

A vaccine efficacy curve provides a useful direct measure of relationship between post-vaccination assay value and vaccine efficacy, offering the potential of directly predicting vaccine efficacy from observation of the post-vaccination assay values of vaccinees.

The question of Which assay? has received less attention in the setting of the licensure of novel and next-generation vaccines in the pharmaceutical industry. In such settings, the mechanism of action of the pathogen is often better understood, as is the intended mechanism of action of the vaccine. Before an efficacy trial is authorized, the US Food and Drug Administration requires demonstration via a 'validated' (i.e. sensitive, specific to the pathogen, reliable, gradable, having minimal inter-technician variation, etc.) assay that the new vaccine has some chance of protecting against disease, and often only a single assay is developed and validated.

3. What assay value?

While the search for an HIV vaccine may be some way from considering the question of what assay value should be used to differentiate susceptible from protected individuals, in the context of the licensure of a novel vaccine in the pharmaceutical industry the quantification of an assay value associated with protection greatly assists subsequent immunological assessment of co-administration with other vaccines, development of combination vaccines, and development and possibly licensure of next generation vaccines. Typically in a vaccine efficacy trial subjects' samples are assayed by the regulator-approved assay, thus together with observations of subsequent disease occurrence providing data for quantifying protection as a function of assay value. (Note: case-cohort design can greatly reduce the number of samples assayed and facilitate testing additional assays [14,10].)

A notable early application of statistical methods to finding a threshold assay value differentiating susceptible from protected individuals was the method used by Chang and Kohberger in the context of protection against invasive pneumococcal disease following vaccination with 7-valent pneumococcal conjugate vaccine [15,16]. The method finds the threshold at which the relative risk of being below the threshold equals the relative risk of disease; i.e. it is the solution to

$$t_p : \frac{\mathrm{P}\left(t < t_p \mid \text{vaccinated}\right)}{\mathrm{P}\left(t < t_p \mid \text{not vaccinated}\right)} = \frac{\mathrm{P}\left(\text{disease} \mid \text{vaccinated}\right)}{\mathrm{P}\left(\text{disease} \mid \text{not vaccinated}\right)}$$

where t = assay value

t_p = threshold differentiating susceptible from protected.

The method may be illustrated by Figure 3. It led to the adoption by the WHO of the 0.35 µg/mL IgG threshold after pneumococcal conjugate vaccination [17], which has also been accepted by other regulators, and was used for the licensure of 13-valent pneumococcal conjugate vaccine [18]. The method has also been used to estimate a

threshold correlate of protection for meningococcal C conjugate vaccine in a sero-epidemiological study [19]. It is a population-based method [20] and does not require individual data on assay value and disease; it is predictive of vaccine efficacy and may be thought of as a population average measure, not explicitly quantifying the level of protection at the threshold. Covariates are not readily introduced, and it assumes the same threshold for vaccinees and non-vaccinees, i.e. it assumes the Prentice (1989) criteria for a valid surrogate endpoint.

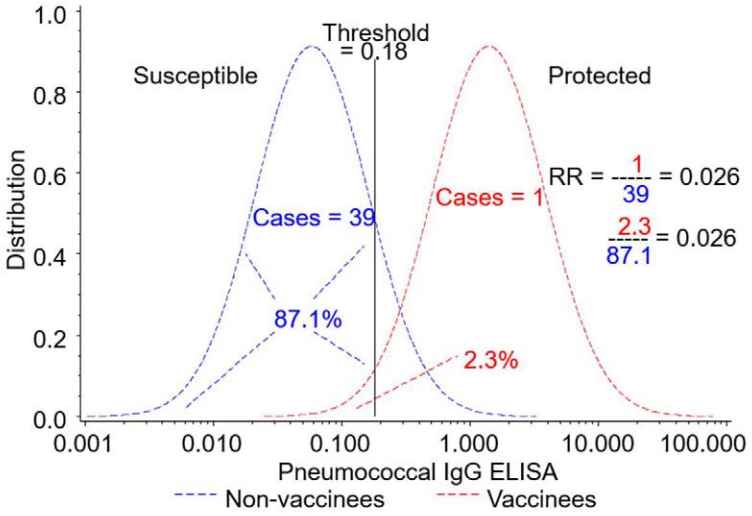

Figure 3. Illustration of method of Chang and Kohberger.

Other methods for finding thresholds include the a:b model, which assumes fixed rates of disease above and below the threshold and selects the threshold maximizing the profile likelihood [21]; the method of Li and Parnes, which finds the threshold that maximizes the correlation between disease status and susceptible/protected status based on assay threshold [22]; and receiver-operating-characteristic based methods [23,24].

Although single-valued thresholds are commonly used to differentiate protected from susceptible individuals, natural variability between individuals means that at any given assay value some individuals will be protected and some susceptible, and if protection does in fact increase with assay value then the level of protection will increase in a smooth continuous manner with assay value, in a 'protection curve', from which values corresponding to 50%, 80% or any other level of protection might be derived. Protection cannot however be measured directly; absence of disease may simply result from absence of exposure. A scaled logit model jointly estimating an exposure parameter and the parameters of a protection curve from assay value and subsequent disease occurrence has been proposed

$$\begin{aligned} \mathrm{P}(\text{disease}) &= \mathrm{P}(\text{exposure}) \times (1 - \mathrm{P}(\text{protected}|\, t)) \\ &= \lambda \times \left(1 - \frac{1}{1+\exp(\alpha+\beta t)}\right) \end{aligned}$$

from which a protection curve conditional on the estimated exposure parameter may be derived [25-27]. The method applied to the White/varicella [28] data is illustrated in Figure 4.

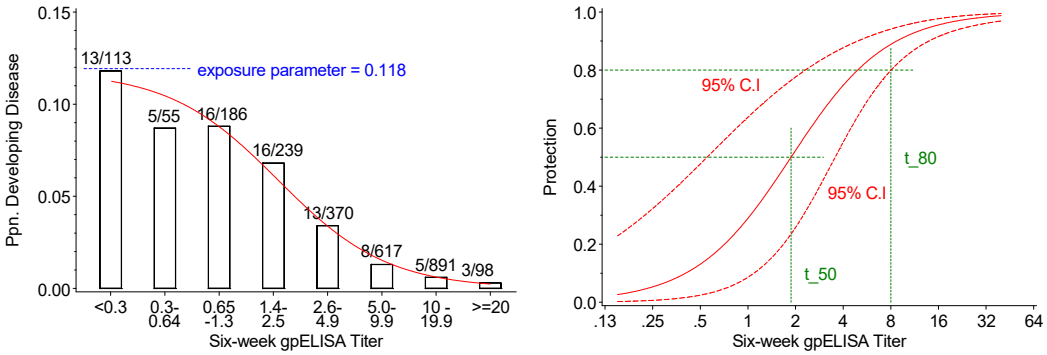

Figure 4. Illustration of the scaled logit model fitted to the White/varicella data showing the fitted model in the left panel, and in the right the derived protection curve with 95% confidence interval for protection and assay values for 50% and 80% protection.

In general, methods for finding thresholds or protection curves should be invariant to the overall rate (incidence) of disease (logistic regression would be a counter-example) and reliable estimates of precision should be available, to assess whether observed differences are due to chance or reflect true differences in the circumstances of each experiment. Consider for example Plotkin's discussion of HAI correlates of protection against influenza, in which point-estimate protective titers of 40, 15, 30, 110, 32 and 64 are contrasted [29]; without estimates of precision it cannot be assessed whether the different titers reflect differences in each experiment or are simply due to chance. The proportion of subjects with assay values within and outside the confidence interval for a protective measure, such as a threshold or the assay value for 50% protection, provides a scale-free evaluation of the precision of the measure, and a gauge of its utility – a measure with a confidence interval spanning most subjects' assay values being of little utility.

4. Terminology

Terminology has evolved in recent years; most recently Plotkin and Gilbert proposed the following [30]:

> Correlate of Protection: An immune marker statistically correlated with vaccine efficacy (equivalently predictive of vaccine efficacy) that may or may not be a mechanistic causal agent of protection
>
> > A correlate of protection can be used to accurately predict the level of vaccine efficacy conferred to vaccine recipients (individuals or subgroups defined by the immune marker level).
>
> Mechanistic Correlate of Protection (aka. Causal agent of protection): A Correlate of Protection that is mechanistically and causally responsible for protection
>
> Non-mechanistic Correlate of protection: A Correlate of Protection that is not a mechanistic causal agent of protection

5. Challenges

The goal for a correlate of protection might be:

An immunological assay reliably associated with protection, and a protective measure (a threshold, or the assay value for 50%, 80%, etc. protection) estimated with sufficient precision that 85%* of subjects are confidently classified as susceptible or protected †.

* or some other high proportion;

† i.e. whose assay values fall outside the 95% confidence interval for the measure.

In addition, other, non-statistical properties are required:

- The assay must be standardized, i.e. use a standardized assay protocol and ingredients, so that different laboratories return the same results on the same samples [31,32];
- The protective measure is defined for a certain population, source of protection (natural infection, vaccination), strain of pathogen, source of challenge (natural infection, experimental) and case definition;
- Ideally, the protective measure should be shown to be consistent across populations, sources of protection, strains of pathogen, sources of challenge and case definitions (as described in the Qin and Gilbert framework above).

5.1 Challenges – quantifying methods

Publications on statistical methodology for quantifying thresholds for protection or assay values for specific levels of protection have so far generally confined themselves to a single population, i.e. no distinction has been made between vaccinees and placebo recipients; (exceptions are the method of Chang and Kohberger, which relies on the differences between vaccinees and placebo recipients to estimate the threshold, and two applications of the scaled logit model [27,33]). Typically, the Prentice criterion is evaluated and not found to be not met at some level of significance, and so data are pooled across treatment groups. A limitation of this approach is that the resulting estimated model is not predictive of vaccine efficacy; small differences between treatment groups in the relationship between assay value and disease result in the predicted efficacy differing from the observed efficacy. This might be problematic from a regulatory perspective, if approval of a vaccine were to be based on a correlate of protection which did not predict efficacy observed. On the other hand, it would appear that for a model to be predictive of efficacy at least one parameter would have to be estimated separately for vaccinees and placebo recipients; so for example there would be one threshold for vaccinees and another for placebo recipients – a not altogether satisfactory conclusion. Further research in this area would be advantageous.

Different thresholds found by different methods can give rise to confusion among non-statisticians. Numerical comparison of the thresholds found by different methods and elucidation of the different interpretations of thresholds would be desirable.

It would seem that not all quantifying methods are equally applicable to all circumstances. For example, when vaccine efficacy is high and assay values of vaccinees and placebo recipients are well separated (difference in log-means >3 standard deviations?) there would seem to be no reason not to use the Chang-Kohberger method. The Qin and Gilbert framework notes that in the context of a correlate of vaccine efficacy vaccination must at a minimum affect assay values and rate of disease; possibly a quantification of these effects could provide a map for when different methods might be appropriate, perhaps as Figure 5. Research in this area would be advantageous.

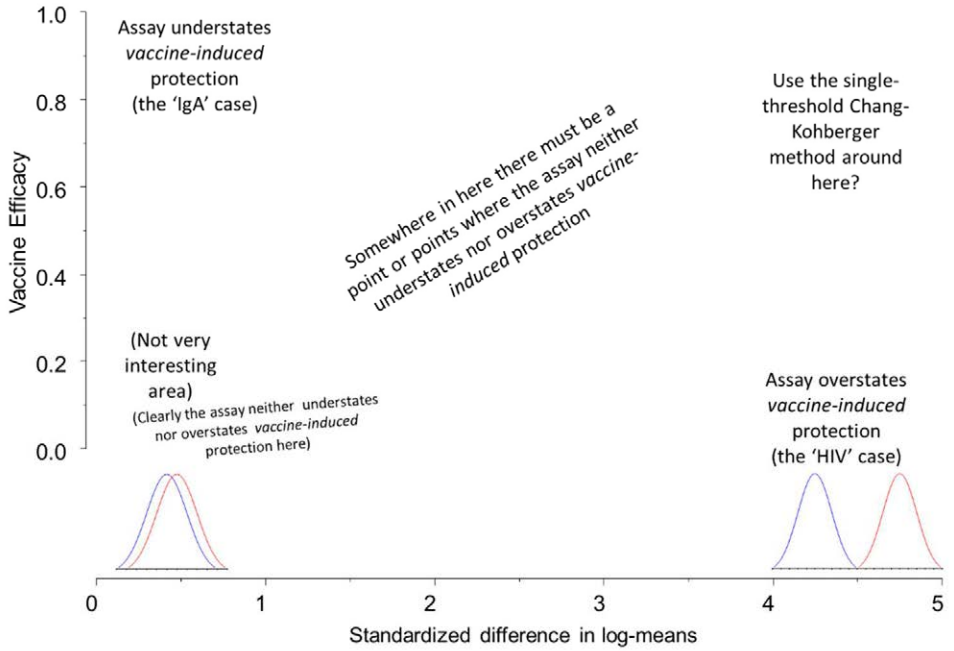

Figure 5. Possible schema for selection of statistical methods quantifying correlates of protection.

5.2 Challenges – assay selection methods

The question of reconciling the Prentice criterion with the prediction of efficacy is alluded to above. Berger has also questioned whether the criterion ensures that the observation of a significant treatment effect on the surrogate endpoint can be used to infer a treatment effect on the true clinical endpoint [34].

The vaccine efficacy curve method requires that the assay values placebo recipients would have had if they had been vaccinated (the 'counterfactuals') be imputed/estimated in order to model how their rate of disease varies with post-vaccination assay value. A question of interest is how to select a predictor and model to consistently estimate the assay values placebo recipients would have had if they had been vaccinated in such a way that the conclusion does not depend on the choice of the predictor/model. Possible criteria being studied are:

- the predictor does not affect disease after accounting for assay value [9,35],
- the predictor is an instrumental variable for disease through assay value [35],
- assay value does not affect disease after accounting for the predictor [35],
- criteria suggested by Long & Hudgens [35,36].

Also of interest would be consideration of whether, if multiple assays were to be imputed, the same predictor/model could be used for all assays. Alternatively, could instead the probability of disease among vaccinees if they had not been vaccinated be imputed/estimated, thus estimating a single counterfactual and allowing standard model-building methods and criteria to be used.

Alternatives to causality might be investigated. It is often said that correlation does not imply causation, and the example given of the cockerel crowing before the sun rises, but not causing the sun to rise. However, for centuries farmers have risen when the cock crows in order to be in the field when the sun rises, not because the cock caused the sun

to rise but because it reliably predicted it would do so. In other words, reliable association may be an alternative to demonstration of causality.

5.3 Challenges – terminology

Although the terminology used may be familiar to those working in the field, it may lead to confusion among those less familiar with the topic. For example, the definition of a correlate of protection as an immune marker statistically correlated with vaccine efficacy suggests that protection and efficacy are synonymous, and does not allow for protection engendered by means other than vaccination. The neophyte notes:

- If the marker is correlated with efficacy, why not call it a correlate of efficacy?
- Natural infection can lead to lifelong immunity, such as is the case with measles, but protection engendered by natural infection would not seem to fall within this definition.

Some well-regarded studies have explored the relationship between antibody and prevention of disease without conditioning on the source of antibody, e.g. Couch/neuraminidase/influenza [37]; clearly such studies should be included in the subject matter of correlates of protection.

Recognition that vaccine efficacy and protection are not synonymous and a better understanding of the distinction between them would improve understanding of the topic. Noting that a popular dictionary defines 'protect' as 'to cover or shield from exposure, injury, damage, or destruction' [38], a more readily understood definition in the context of disease occurrence might be: Protection is a property such that if exposed to the pathogen disease does not occur. Vaccine efficacy would then be given by

$$\mathrm{VE} = 1 - \frac{1 - \mathrm{P}(\text{protected} \mid \text{vaccinated})}{1 - \mathrm{P}(\text{protected} \mid \text{not vaccinated})}.$$

The term 'validated' is used in two senses. In a regulatory context a validated assay is one that has been approved by a regulatory agency for use in later phase clinical trials; in other settings it is used to signify meeting some criterion or test, such as the Prentice criterion. Further, use in the latter sense can lead to confusion by suggesting that assays either are or are not validated, whereas in actuality statistical results are only demonstrated up to a certain level of statistical significance. Similarly, 'finding a correlate of protection' is sometimes heard spoken of, which is no more a proper description of a scientific result than recent popular news reports suggesting that a Higgs boson had been 'found'; CERN reported that the probability of seeing the result observed if a Higgs boson did not exist was 1.7×10^{-9} [39].

6. Summary

The impact of vaccines on global health is second only to that of clean drinking water [40], and vaccines have been found for many prevalent and morbid diseases. However, principally those discoveries have been where the mechanism of action has been more readily understood and future vaccines will likely target diseases less well understood, such as HIV, less prevalent, and emerging pandemics. Such targets may be less amenable to demonstration of vaccine efficacy by clinical endpoint efficacy trials, and may rely more on immunological assays for demonstration of effectiveness. Statistical methods for identifying and quantifying such assays have advanced in recent years, but more remains

to be done if the goal of demonstrating by statistical means assays reliably associated with protection and with adequately precise measures of protection is to be achieved.

Acknowledgements

We would like to thank the various researchers we have collaborated with on different research projects, and numerous colleagues and acquaintances for helpful discussion.

In particular, we would like to thank Dr. Peter Gilbert of the Fred Hutchinson Cancer Research Center for his review of this paper and comments and suggestions which lead to significant improvements in the work.

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