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A model for immunological correlates of protection

Andrew J. Dunning*,†

Wyeth Vaccines Research, 401 N. Middletown Road, Pearl River, NY 10965, U.S.A.

SUMMARY

Immunological assays measure characteristics of the immune system, such as antibody levels, specific to certain diseases. High assay values are often associated with protection from disease. A question of interest is how the relationship between assay values and subsequent development of disease should be quantitatively modelled. Existing approaches successfully model the relationship for high assay values, where the probability of developing disease is low. However at low assay values, the probability of developing disease is more closely associated with factors such as disease prevalence rates and an individual's chance of exposure to infection; these are less well captured by existing models. This paper presents a model that accommodates both assay values and factors independent of assay values, enabling protection from disease to be modelled over the whole range of assay values and proposing a method for predicting the efficacy of a vaccine from the assays of vaccinees and non-vaccinees.

KEY WORDS: vaccine; correlate of protection; correlate of immunity; surrogate endpoint; immunological assay

1. INTRODUCTION

Vaccines prevent disease by stimulating an immune response similar to that produced by the disease-causing microorganism itself. They present a weakened or inactivated form of the microorganism, or a fragment of the microorganism, to the body. The immune system responds by activating a system of macrophages, T-cells and B-cells to form antibodies and attack and eliminate the pathogen. Following the immune response, the persistence of antibodies and immunological memory enables the body to quickly recognize and destroy the disease-causing microorganism if it invades the body again.

*Correspondence to: Andrew J. Dunning, Wyeth Vaccines Research, 401 N. Middletown Road, Pearl River, NY 10965, U.S.A.
†E-mail: adunning@alumni.washington.edu

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The efficacy of a vaccine is measured by the extent to which it prevents disease in vaccinated individuals. Vaccine Efficacy is estimated by

$$\mathrm{VE} = 1 - \frac{r_V}{r_P} \tag{1}$$

where r_V is the rate of disease among vaccinated individuals and r_P is the rate among unvaccinated individuals. VE has a convenient interpretation as the magnitude of the reduction in disease as a result of vaccination. To estimate the efficacy of a new vaccine, a placebo-controlled clinical trial is often conducted, and the rate of disease among vaccinated and placebo-receiving subjects is measured.

If however an improved form of the vaccine is later formulated, or a new manufacturing process developed, or it is wished to study the effect of administering other vaccines at the same time, a placebo-controlled trial could now be considered unethical, since accepted means of preventing the disease already exist. Also, many diseases that vaccines prevent are comparatively rare, requiring large clinical trials. A method of estimating the efficacy of a vaccine other than by a large clinical trial is desirable.

A possible alternative to an efficacy trial of a new formulation is to find an assay that measures an aspect of the immune system that correlates with protection from disease. Then, a smaller trial could be conducted, the immune response induced by the new vaccine formulation assayed, and the efficacy of the new vaccine could be predicted if the quantitative relationship between assay values and protection from disease had been previously established.

Many assays have been developed which measure antibody levels or some other aspect of immune response. Serum bactericidal assays quantify the ability of a subject's serum to kill the disease-causing microorganism *in vitro*. Certain pathogens cause red blood cells to stick together, or agglutinate; hemagglutination inhibition assays measure the ability of antibodies generated by the immune system to prevent agglutination. Enzyme-linked immunosorbent assays (ELISA) measure the extent to which antibodies in a subject's serum bind to the microorganism or its parts. Other examples could be given; in each case, the result is some measure of immune response specific to the disease of interest.

The statistical question of interest is how the relationship between assay values and protection from disease should be modelled. A traditional approach has been to look for a threshold level of assay value above which subjects do not develop disease. Individuals who achieve the threshold following vaccination are considered protected. Many studies have adopted this approach [1–4]. A limitation of this model is that the relationship between assay values and the occurrence of disease below the threshold level is usually not specified. Thus, in general, the model does not provide a method of predicting vaccine efficacy, which is estimated from the occurrence of disease. In addition, individuals with assay values above a chosen threshold will occasionally develop disease, complicating the task of determining a consistent threshold level.

Logistic regression has been used to quantify the relationship between assay values and the occurrence of disease. Piedra *et al*. studied patients hospitalized with acute respiratory infection. They used logistic regression to show that the odds of hospitalization being due to respiratory syncytial virus were statistically significant for each of three assays [4]. Storsaeter

et al. used logistic regression to model pertussis illness as a function of pertussis antibody values [5]. Chan *et al*. fitted Weibull, log-normal, log-logistic and piecewise exponential models to cases of varicella among vaccinated children. They found that glycoprotein ELISA assay results 6 weeks after vaccination were strongly correlated with long-term protection against disease [6]. In the context of HIV vaccine trials, Hudgens *et al*. used truncated distributions to model vaccine effects on HIV viral loads [7]. Siber observed that the selection of a protective threshold can be arbitrary, and suggested that the level of protection likely varies as a continuous function of the antibody level; he proposed the assessment of titre-specific protection and gave a formula for calculating titre-specific rates of disease [8]. However, methods for using associations found by these approaches to directly predict vaccine efficacy from assay values have not been forthcoming.

Existing models successfully capture the observed relationship between immunological assay values and protection from disease for high assay values. At high values few people develop disease, so it may be taken as axiomatic that individuals with high values are protected. But at low assay values, some people develop disease and some do not. Many of the diseases that vaccines prevent are infectious, that is, acquired by exposure to an infected person. The rate of disease among individuals with low assay values may be more strongly associated with the chance of exposure and disease prevalence rates, which vary from year to year and place to place. At low assay values, the rate of disease may be largely determined by factors independent of assay values.

The proposed model therefore separates the effect of assay values from factors independent of assay values. The model is described in Section 2 using commonly made simplifying assumptions. Various features and applications are given in Section 3. In Section 4, the model is illustrated with data from eight assays from a clinical trial of pertussis vaccines in Germany in 1991–1994. Section 5 discusses the results and presents models based on alternative assumptions. Limitations of the model and directions for further research are also discussed in this section.

There are many clinical and technical problems involved in developing assays that correlate well with protection from disease. Immune responses may differ in different populations or according to the time after vaccination when serum samples are taken. Difficulties in performing assays may result in imprecision in assay values. The model presented here does not attempt to resolve all such issues; rather it provides a quantitative framework within which such questions might be addressed.

2. THE MODEL

Suppose in a clinical trial serum samples are taken from subjects, and in a subsequent surveillance period the occurrence or non-occurrence of disease in each subject is recorded. The serum samples are assayed and assay values obtained. Assume there are data from n subjects, indexed $i = 1, \ldots, n$. Let

t represent a subject's assay value, and

$y = 1$ represent the event that they develop disease, $y = 0$ that they do not

The data thus consist of n pairs $\{y_i, t_i\}$. Assay values may be titres or concentrations, and for convenience the term titre will be used here to include both. t represents the assay value after any transformations have been applied; it is customary to take logs of assay values in immunological calculations and it will be assumed that this has been done in this case, so that t can take both positive and negative values.

Initially it is assumed that an individual cannot develop disease more than once during the surveillance period, that the surveillance period is equal for all subjects, and that the immune system operates so as to render individuals either protected from or susceptible to disease. Thus protected individuals are completely immune from disease, while susceptible individuals constitute a homogenous group all equally at risk; there are no gradations of immunity. Let

$\pi(t)$ represent the probability that an individual with titre t is protected

or equivalently, $1 - \pi(t)$ is the probability that an individual is susceptible. $\pi(t)$ will be modelled as a strictly increasing smooth function of t mapping into $(0, 1)$. Let

λ represent the probability that a susceptible individual develops disease

λ will depend on factors specific to the clinical trial—the chance of exposure, the prevalence of disease and other factors independent of assay values.

The probability that a subject develops disease is the probability that the subject is susceptible multiplied by the probability that susceptible individuals develop disease,

$$P(Y_i = 1) = \lambda(1 - \pi(t_i)) \tag{2}$$

There are a number of possible functions by which $\pi(t)$ may be modelled. Alternative choices are discussed in Section 5; for the present, the two-parameter inverse logit function will be used. That is,

$$\pi(t_i) = \frac{\exp(\alpha + \beta t_i)}{1 + \exp(\alpha + \beta t_i)} \tag{3}$$

Thus for small assay values, $\pi(t)$ approaches 0; and as t gets large, $\pi(t)$ approaches 1. Combining (2) and (3) gives the probability that a subject develops disease,

$$P(Y_i = 1) = \frac{\lambda}{1 + \exp(\alpha + \beta t_i)} \tag{4}$$

and the likelihood is

$$\mathrm{L}(\lambda, \alpha, \beta; \{y_i, t_i\}) = \prod_i \left(\frac{\lambda}{1 + \exp(\alpha + \beta t_i)} \right)^{y_i} \left(1 - \frac{\lambda}{1 + \exp(\alpha + \beta t_i)} \right)^{(1 - y_i)} \tag{5}$$

λ, α and β may be estimated by standard maximum likelihood methods. The Newton–Raphson algorithm was used to fit the model. The model will be referred to as a scaled logit model.

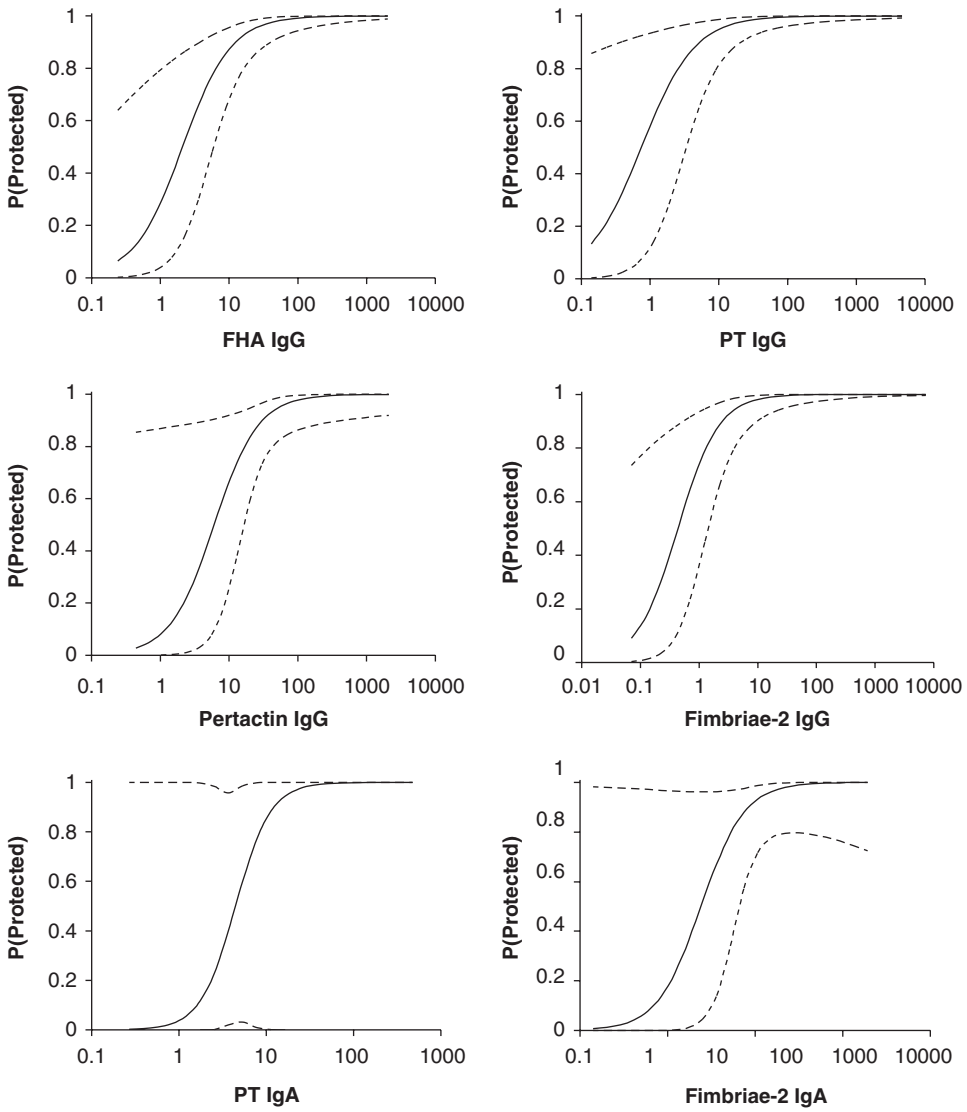

Figure 1. Probability of protection as a function of assay value for six pertussis assays using data from the German pertussis vaccine trial, together with 95 per cent two-sided confidence intervals. Assay values are shown returned to their natural units of measurement, on a logarithmic scale; for calculations, logs of assay values were used.

The components of the model, $\pi(t)$ and λ, are illustrated graphically in Figures 1 and 2. The model has similarities to certain dose–response models; see for example Setzer *et al.* [9]. The algebraic derivation of the scores and the information, and SAS code to implement the fitting, are available at http://stats.adunning.net.

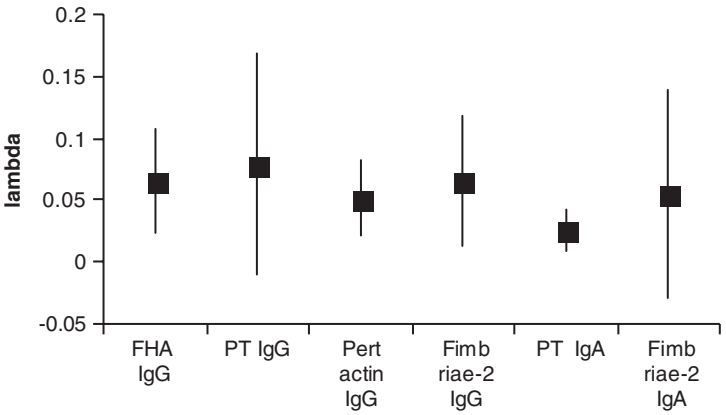

Figure 2. Point estimates and 95 per cent confidence intervals for λ, the probability of developing disease if susceptible, for six assays in the German pertussis vaccine trial.

3. FEATURES OF THE MODEL

The model completes the unspecified part of the threshold model. In both models, the probability of developing disease is zero or close to zero for assay values above the threshold. In the scaled logit model, the probability of developing disease increases with decreasing assay values, until at the lowest assay values it approaches the rate of disease among susceptible individuals.

The model follows Siber's suggestion to model protection as a continuous function of assay values, but refines his titre-specific rate of disease to differentiate between titre-specific protection, $\pi(t)$, and the rate of disease among unprotected individuals, λ. A specific parametric function, the inverse logit function, is proposed for the continuous function of assay values.

The parameters α and β are the location and scale parameters of the inverse logit function. For low values of β the value of the function increases slowly with t, and thus correspond to situations where the probability of protection increases only slowly over the range of assay values. High values of β correspond to the probability of protection increasing sharply at an assay value of $-\alpha/\beta$. In the limiting case, the model approaches the threshold model; for large β the probability of protection approaches 1 for assay values above $-\alpha/\beta$.

The probability that an individual is protected from disease may be estimated from estimates of the model parameters. For an individual with titre t, the probability that they are protected is given by $\hat{\pi}(t) = \exp(\hat{\alpha} + \hat{\beta}t)/(1 + \exp(\hat{\alpha} + \hat{\beta}t))$. Plots of the probability of being protected *versus* assay values for the assays in the pertussis vaccine trial are shown in Figure 1.

A confidence interval for the probability that an individual with titre t is protected from disease may be calculated. The asymptotic variance–covariance matrix of $\hat{\lambda}$, $\hat{\alpha}$ and $\hat{\beta}$ is the inverse of the expected information matrix. This may be estimated by the inverse of the observed information matrix, which can be constructed from quantities computed by the Newton–Raphson fitting algorithm. Using estimates of the variances and covariance

of $\hat{\alpha}$ and $\hat{\beta}$, the variance of $(\hat{\alpha}+\hat{\beta}t)$ may be calculated as a function of t

$$\mathrm{Var}(\hat{\alpha}+\hat{\beta}t)=\mathrm{Var}(\hat{\alpha})+t^2\,\mathrm{Var}(\hat{\beta})+2t\,\mathrm{Cov}(\hat{\alpha},\hat{\beta}) \tag{6}$$

Thus limits for a confidence interval for $(\hat{\alpha}+\hat{\beta}t)$ may be calculated, and since the inverse logit is a strictly increasing function of its argument, a confidence interval for the probability of an individual being protected may be constructed from the inverse logits of the limits of the confidence interval for $(\hat{\alpha}+\hat{\beta}t)$. Confidence intervals constructed in this manner are illustrated in Figure 1.

Subject to the limitations discussed in Section 5, the model provides a method of predicting the efficacy of a new vaccine formulation from the assay values of subjects. Suppose $\hat{\alpha}$ and $\hat{\beta}$ have been estimated in a previous clinical trial in which both the subjects' assay values and the occurrence of disease were measured. A smaller trial of a new vaccine formulation might be conducted in which the assay values of subjects in a vaccinated group (V) and a placebo group (P) are measured, in a similar manner to the previous trial. The expected number of subjects who would develop disease in the vaccinated group would be, from (4)

$$\sum_{i\in V} P(Y_i=1)=\sum_{i\in V}\frac{\lambda'}{1+\exp(\hat{\alpha}+\hat{\beta}t_i)} \tag{7}$$

where λ' is the unknown probability of disease among susceptible subjects in the new trial. The expected number of subjects who would develop disease may be calculated similarly for the placebo group P. Thus expected disease rates may be calculated. In the computation of vaccine efficacy the λ's would cancel, and the efficacy of the new formulation would be predicted by

$$\mathrm{VE}_{\mathrm{new}}=1-\frac{1/n_V\sum_{i\in V}1/\left(1+\exp(\hat{\alpha}+\hat{\beta}t_i)\right)}{1/n_P\sum_{i\in P}1/\left(1+\exp(\hat{\alpha}+\hat{\beta}t_i)\right)} \tag{8}$$

4. ILLUSTRATION OF APPLICATION OF THE MODEL

The model may be illustrated with data from a controlled clinical trial to assess the efficacy of two pertussis vaccines that was conducted in Germany between 1991 and 1994 [10]. Ten thousand two hundred and seventy one healthy infants aged 2–4 months received either diphtheria–tetanus–whole cell pertussis vaccine (DTP), diphtheria–tetanus–acellular pertussis vaccine (DTaP) or diphtheria–tetanus only vaccine (DT). Subjects received three doses of DTP or DTaP vaccine or two doses of DT vaccine before 6 months of age and a booster dose at 15–18 months. Subsequently, they were followed for an average of 2.07 years and cases of pertussis recorded. Overall, the efficacy of the DTP vaccine was found to be 93 per cent and of DTaP 83 per cent, relative to DT, in the per-protocol population based on the primary case definition for pertussis.

Serum samples were collected following vaccination and during the acute and convalescent phases of possible pertussis illnesses. Each sample was assayed for immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies to filamentous hemagglutinin (FHA), pertussis toxin (PT), pertactin and fimbriae-2, making in total eight assays.

A subset of 2003 subjects was randomly selected to provide additional samples during the surveillance phase to investigate the kinetics of serum antibody levels. We used the assay results from these subjects to estimate the association between immunological assay values and protection from subsequent pertussis. Of the 2003 subjects, 50 developed disease. Six however received a booster vaccination between the serum sample being taken and the onset of illness and were therefore excluded, leaving 44 subjects who developed pertussis and 1953 who did not.

The models were fitted as described above, after taking logs of the assay values. For two of the assays, the fitting algorithm did not converge; for the remaining six, the parameter estimates and selected variance–covariance estimates are shown in Table I.

The fitted curves of the relationship between assay values and the probability of protection are shown in Figure 1. For all six assays that converged, the estimated probability of protection increased from near zero to close to one over the range of the assay values. Typically, there was a range of high assay values for which the estimated probability of being protected from disease exceeded 0.95, and for three assays the lower limit of the 95 per cent confidence interval also exceeded 0.95 over most of this high range. For four assays—the IgG assays—the confidence intervals were narrow at high assay values and broadened considerably at low assay values.

For the two IgA assays for which the fitting algorithm converged, the confidence intervals were considerably wider. The upper limit was close to one over the whole range of values for both assays, and the lower limit was close to zero over the whole range for the PT IgA assay.

In addition to samples taken from the 2003 subjects above, serum samples were also collected from subjects at the onset of suspected pertussis. The assay values from these samples were used with the parameter estimates previously derived to predict vaccine efficacy among these subjects. Since pertussis antibodies peak shortly after vaccination or illness and subsequently decline, samples taken less than 90 days after vaccination or more than 4 days after the onset of pertussis-like symptoms were excluded. There were 906 subjects with samples meeting these criteria, of whom 29 subsequently developed pertussis. Vaccine efficacy was predicted in the DTP and DTaP groups relative to the DT group using the subjects' assay values and the parameter estimates previously obtained, using (8), and compared to the actual vaccine efficacy in these two groups relative to DT based on disease occurrence rates, using (1). Table II shows the results.

Table I. Estimates of parameters of the scaled logit model for eight pertussis assays using data from the German pertussis vaccine trial [10, 11].

Assay	$\hat{\lambda}$	$\hat{\alpha}$	$\hat{\beta}$	$\mathrm{Var}(\hat{\lambda})$	$\mathrm{Var}(\hat{\alpha})$	$\mathrm{Var}(\hat{\beta})$	$\mathrm{Cov}(\hat{\alpha},\hat{\beta})$
FHA IgG	0.0658	−0.921	1.239	0.00046	1.338	0.155	−0.393
PT IgG	0.0787	0.325	1.122	0.00208	1.419	0.159	−0.375
Pertactin IgG	0.0516	−2.449	1.359	0.00025	4.908	0.407	−1.361
Fimbriae-2 IgG	0.0653	1.046	1.261	0.00070	0.679	0.176	−0.180
FHA IgA	did not converge						
PT IgA	0.0251	−3.240	2.171	0.00007	38.426	17.078	−24.638
Pertactin IgA	did not converge						
Fimbriae-2 IgA	0.0560	−1.549	1.762	0.00184	6.319	1.180	−2.574

Table II. Illustration of the prediction of vaccine efficacy from immunological assay values for two pertussis vaccines, compared to efficacy calculated from disease occurrence rates, in the German pertussis vaccine trial.

	Treatment Group		
	DT $N = 188$	DTP $N = 354–362$	DTaP $N = 345–356$
Efficacy from occurrence rates			
Subjects developing pertussis—number	18	4	7
—proportion	0.096	0.011	0.020
Vaccine efficacy relative to DT		88.4%	79.4%
Efficacy predicted from assay values			
FHA IgG		53.9%	85.4%
PT IgG		77.4%	48.9%
Pertactin IgG		71.2%	82.5%
Fimbriae-2 IgG		72.6%	56.3%
PT IgA		0.0%	−2.2%
Fimbriae-2 IgA		24.1%	15.5%

Two of the predictions of vaccine efficacy estimated from assay values were within 10 percentage points of the efficacy calculated from disease occurrence rates. In both of these cases—efficacy of DTaP predicted from FHA IgG assay values and of DTaP from Pertactin IgG assay values—the predicted efficacy was higher than the efficacy based on occurrence rates. All other efficacy predictions from assay values were lower than those based on occurrence by more than 10 percentage points. Some of the assay values in this data came from samples taken after pertussis symptoms first appeared and may thus be inflated by an immune response to the illness, resulting in the efficacy predicted being lower than it would otherwise have been.

5. DISCUSSION

For five of the eight assays, the Newton–Raphson fitting algorithm converged quickly, typically stabilizing to the values shown in Table I in less than ten iterations. For the PT IgA assay it was necessary to try different starting values, and to reduce the size of the iterative steps and increase their number in order to achieve convergence. For the assays that did not converge, it was noted that estimates moved towards values of the parameters outside of the plausible parameter space, such as estimates for λ of 0.8, before quantities in the computation exceeded machine limits.

The assays that converged most readily were in general those with the least variance as shown in Figure 1. The widest confidence band for the probability of protection was for the PT IgA assay, which was the assay most difficult to fit. It might be hypothesized that ease of fitting, low parameter variance and narrower confidence intervals correspond to assays that are ‘good’ correlates of protection. If this were so, it would suggest that the four IgG assays were useful correlates, the Fimbriae-2 IgA assay was a fair correlate, PT IgA was a weak correlate, and the FHA IgA and Pertactin IgA assays were not useful as correlates of protection in terms of this model. These suggestions may be compared with the findings of

Cherry *et al*. [11], who, based on data from the same study, found that among the IgG assays the mean PT, Pertactin and Fimbriae-2 antibody values were higher among non-cases than cases, and that only PT and Pertactin were significant in protection in a multivariate (classification tree) analysis. It should be noted however that the results in Table I and Figure 1 are intended to illustrate the statistical method and are not intended to be scientifically valid findings. There are a number of known features of the immunology of pertussis, such as decreasing antibody levels over time, which are not taken into account in these results.

It may be seen from Figure 1 that the variance of the estimated probability of being protected is consistently greater at low assay values than at high. Two possible reasons may be suggested for this. Firstly, at low assay values the model must estimate both λ and the parameters of the inverse logit, whereas at higher assay values the parameter λ has less influence; λ represents the probability of developing disease if susceptible, and the probability of being susceptible at high assay values is low.

Secondly, low assay values tend to be measured with less relative precision than high values, thus tending to increase the variance of the parameter estimates at lower values. Often, laboratories provide a minimum quantifiable limit for assay values below a certain cut-off. Taking logs of assay values increases the effect of lack of precision at low values.

If λ is interpreted as the probability that a susceptible individual develops disease, then it might be expected that the estimates of λ for different assays on the same samples in the same trial would be close together. The point estimates and confidence intervals for λ for the six pertussis assays which converged are shown in Figure 2. The estimates do not lend strong support to the suggestion that λ represented the same quantity for all six assays. It may be noted that the low outlier for this estimate, the PT IgA assay, was also the assay for which the confidence interval for the probability of protection was close to $(0, 1)$ over the whole range of assay values, confirming the conclusion that this assay was a poor correlate of protection. The estimate of λ for this assay, 0.0251, was only slightly higher than the overall disease rate of 0.0228 for these subjects. Taken together, these arguments support the interpretation of λ as representing the probability of developing disease amongst individuals without protection *arising from the aspect of the immune system measured by the assay*, that is, as representing effects that are independent of assay value, while $\pi(t)$ represents effects that are a function of assay value.

Other possible smooth increasing functions were considered for $\pi(t)$; the effect of using a cumulative normal distribution function instead of the inverse logit function was examined by fitting both functions to the FHA IgG data. The difference in the estimated probability of protection under the two models was less than 0.015 at all points in the range of assay values. Further, using the cumulative normal distribution function does not lead to a straightforward method for constructing confidence intervals for the probability of protection, as does the inverse logit model. The logit function is also the canonical link function for binary data, and the scaled logit model would in fact approach standard logistic regression if λ approached 1. These arguments suggest that there would be little advantage to using some other symmetric parametric function for $\pi(t)$.

The model parameters are estimated by maximum likelihood, and hence the parameter estimates have the optimality properties of maximum likelihood estimators—consistency, asymptotic efficiency and asymptotic normal distribution. By the invariance property of maximum likelihood estimators under functional transformations, the estimated probability of protection is also a maximum likelihood estimator with similar properties [12].

Part of the appeal of the threshold model is that the relationship between assay values and protection can be characterized by a single number. Two parameters are required to describe the logit relationship. However, single-valued measures could also be derived; the assay value at which 90 per cent of individuals were protected against disease might be one such measure.

The model presented in Section 2 assumes that surveillance is equal for all individuals; more general models accommodating unequal surveillance may be developed using hazard rates. Smith, Rodrigues and Fine suggest two models for vaccine efficacy based on hazard rates, depending on the assumed mechanism of action of the vaccine [13]. If vaccination is assumed to reduce the hazard of disease uniformly for all vaccinees, then the probability of disease for vaccinees is $1-\exp[-(1-\mathrm{VE}_1)\lambda'' u_i]$, where λ'' represents the hazard rate among unvaccinated individuals, VE_1 represents the proportionate reduction in hazard rate for vaccinees and u_i represents a subject's surveillance time. Alternatively, if vaccination is assumed to render some proportion of vaccinees protected from disease while leaving the remainder susceptible, then the probability of disease for vaccinees is $(1-\mathrm{VE}_2)[1-\exp(-\lambda'' u_i)]$, where VE_2 represents the proportion of previously susceptible individuals rendered protected through vaccination.

Parallel models for the effect of assay values on the development of disease may be stated. If it is assumed that a proportion of individuals with a certain titre are protected from disease and the remainder susceptible, the probability of disease may be modelled as

$$P(Y_i = 1) = (1 - \pi(t_i))(1 - \exp(-\lambda''' u_i)) \tag{9}$$

where λ''' is the hazard rate for susceptible individuals. Vaccine efficacy, that is, Smith, Rodrigues and Fine's VE_2, could be predicted using parameter estimates from this model and (8). When surveillance is equal for all subjects, (9) is equivalent to (2).

Alternatively, if a certain titre is assumed to reduce the hazard rate uniformly for all individuals with that titre, the probability of disease may be modelled as $1 - \exp[-(1 - \pi'(t_i))\lambda''' u_i]$, where $\pi'(t)$ represents the proportionate reduction in hazard rate for an assay value of t. However, Smith, Rodrigues and Fine's VE_1 may now only approximately be estimated by (8).

The parameter estimates from the pertussis trial (Table I) and the prediction of vaccine efficacy (Table II) are based on an equal surveillance model. Preliminary results from a model taking unequal surveillance into account suggest that the general conclusions as to ease of fitting, variance of estimated quantities and predicted vaccine efficacy are not substantially changed when surveillance is taken into account.

A limitation of the model as presented is that it is set in an undifferentiated population; it assumes that the relationship between assay values and protection is the same for different subgroups. In particular, the prediction of vaccine efficacy at (8) assumes that $\pi(t)$ is the same for vaccinees and placebo-recipients, that is, that the relationship between assay values and protection is independent of vaccination status. While this might be true for an ideal correlate of protection, there are several reasons why it might not be so in practice.

Firstly, it is known that the effects of the immune system are mediated through several different pathways of action and it is unlikely that a given assay will capture the full effect of them all. In addition, the immune response to many vaccines is qualitatively different from that elicited by natural infection; subunit vaccines for example, contain only part of the disease-causing pathogen. Further, in many immunological studies the assay values measured in vaccinees are not intended to be comparable to those of placebo recipients with respect

to longer term protection. Rather, assay values of vaccinees are often measured at what is believed to be the peak of the immune response to vaccination, as an indication of which vaccinees have responded immunologically to the vaccine and which not, the assay values of placebo recipients being merely a reference level.

Less well understood however is whether and to what extent known immunological differences between vaccinees and non-vaccinees translate into quantitative differences with respect to the relationship between their assay values and protection. For a given assay value, do vaccinees have more protection than placebo recipients or less? If an assay could be found that under certain circumstances predicted protection independent of vaccination status, it might be considered an ideal correlate of protection. The model presented provides a framework within which such questions might begin to be addressed.

Further research might therefore examine how the consistency or otherwise of the relationship between assay values and protection might be evaluated across subgroups. In some instances, it might be possible to conclude that the relationship between assay values and the development of disease was independent of vaccination status by inspection of separate estimates of model parameters among vaccinees and non-vaccinees in the same study, but a rigorous comparison would necessitate the development of a statistical test of the hypothesis that the two groups came from the same population.

Comparisons across studies would also be of interest. If disease rates differed in similar populations in different studies, a test showing that the protection mediated through assay values was the same in each population—that is, that differences in $\hat{\alpha}$ and $\hat{\beta}$ in the two studies were not statistically significant—would validate the assay as a correlate of protection and imply that the differences in disease rates were attributable to factors independent of assay values.

Further research might also examine how the variance of the estimates of α and β could be brought into the estimate of vaccine efficacy at (8). Investigation of the validity of the asymptotic variance estimates might also be valuable.

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