Module 8
Evaluating Immunological Correlates of Protection

Session 3
Evaluating Correlates of Protection Using Individual, Population, and Titer-specific Approaches

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Individual-Based Correlates of Protection
Identification of Protective Level by Looking at Vaccine Failures
Individual-Based Correlate of Protection

- Assess antibody titers after immunization or just before infection and determine disease occurrence afterwards.
- Identify “protective level” of antibody:
  - 100% protection for individuals who have protective levels of antibody.
  - Vaccine failures (who develop breakthrough disease) must have lower titers.
  - Compare antibody titers in vaccine failures and protected individuals.

Measles Example

Chen et al. JID 1990

- A measles outbreak with 112 cases occurred in Boston area in 1985.
- 100 cases were in Boston University students:
  - 40 were in a 3-tower dormitory complex.
- The American Red Cross had held a blood drive at BU during that time:
  - Allowing preexposure blood specimens to be obtained from cases and noncases.
**Measles Case Definition**

- Generalized maculopapular rash of $\geq 3$ days duration
- Fever $\geq 38.3^\circ C$, and
- At least one of the following symptoms:
  - Cough
  - Coryza
  - Conjunctivitis

**Reported Measles Cases By Date of Rash Onset, Boston University**

Chen et al. JID 1990
Serologic Testing

- Blood samples were available for 80 participants in this study
  - 8 cases and 72 noncases
- Plaque Reduction neutralization (PRN) test
  - 2 or 4-fold dilutions of serum starting at 1:8
  - PRN titer defined as the serum dilution that would reduce the number of plaques by 50%
- EIA

### PRN Titers of Cases and Noncases

<table>
<thead>
<tr>
<th>Subject(s)</th>
<th>PRN titer</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-exposure</td>
<td></td>
<td>Post-exposure</td>
<td>IgM</td>
</tr>
<tr>
<td>Cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>&lt;16</td>
<td>35,363</td>
<td>&lt;160</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>17,723</td>
<td>&lt;102</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>39,268</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>86</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>86</td>
<td>101,339</td>
<td>&lt;48</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>98</td>
<td>44,661</td>
<td>662</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>118</td>
<td>14,157</td>
<td>509</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>120</td>
<td>13,638</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Total, %</td>
<td></td>
<td>&lt;1052</td>
<td>&lt;1052</td>
<td></td>
</tr>
<tr>
<td>Noncases, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 37</td>
<td>&lt;1052</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 35</td>
<td>1052</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Geometric Mean Titer (GMT) = 63 vs 1157 for cases vs noncases (p<.001)

Chen et al. JID 1990
Protective PRN Titer of 120

- Highest preexposure titer among cases
- 8 out of 9 students with a PRN titer ≤120 had measles
- 0 out of 71 with a PRN titer >120 had measles
- Fisher’s exact test: P<.0001
- GMTs in symptomatic noncases (871) significantly lower than asymptomatic students (1549), P<.04

EIA Results

- No cases had detectable antibody vs. a GMT of 183 for noncases
- 3 out of 72 noncases had undetectable antibody titers
- GMTs in symptomatic noncases (153) not statistically significantly different from asymptomatic students (220), P=.10
Summary of the Measles Example

- PRN titer of >120 indicates protection
  - Presence of low PRN titers may not offer protection
- PRN assay is more sensitive than EIA
- Protective titer identified by assessing individual-based titer levels among vaccine failures

Problems with Individual-Based Correlates of Protection

- The level of antibody required to protect a given individual against a particular exposure is likely to vary. Other factors also likely to affect outcome.
- Clear cut threshold may not exist for 100% protection as breakthrough disease may occur in individual with high titers
- Difficult to implement prospective study design as it requires taking blood samples from a large number of participants, particularly when disease incidence rate is low
Diphtheria Example
(Siber, DBS 1997)

- Diphtheria antitoxin titer of >0.01 AU/ml is presumed to indicate protection in population surveys
  - Produce a negative Schick test (skin test for diphtheria)
- Ipsen studied diphtheria in an outbreak in Copenhagen in 1943-1944
  - 106 patients had been immunized (vaccine failures)

Diphtheria Antitoxin Titers
(Siber, DBS 1997)

A. Breakthrough Diphtheria Cases

<table>
<thead>
<tr>
<th>Titer Range</th>
<th>% of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.1</td>
<td>4.7%</td>
</tr>
<tr>
<td>0.1-1</td>
<td>13%</td>
</tr>
<tr>
<td>1-10</td>
<td>32%</td>
</tr>
<tr>
<td>&gt;10</td>
<td>56%</td>
</tr>
</tbody>
</table>

B. Healthy Immunized Population

<table>
<thead>
<tr>
<th>Titer Range</th>
<th>% of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.1</td>
<td>2.8%</td>
</tr>
<tr>
<td>0.1-1</td>
<td>12.1%</td>
</tr>
<tr>
<td>1-10</td>
<td>25%</td>
</tr>
<tr>
<td>&gt;10</td>
<td>66%</td>
</tr>
</tbody>
</table>
Diphtheria Example

- The antitoxin titers overlap between breakthrough cases and healthy immunized population
- However, Ipsen noted a strong negative correlation between the level of antitoxin and severity of diphtheria

<table>
<thead>
<tr>
<th>Antitoxin (AU/ml)</th>
<th>Risk of complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.01</td>
<td>33%</td>
</tr>
<tr>
<td>0.1 to 1.0</td>
<td>6.7%</td>
</tr>
<tr>
<td>&gt;1.0</td>
<td>5.4%</td>
</tr>
</tbody>
</table>

Pertussis Example

Swedish Acellular Pertussis Vaccine Trial in 1985-86

- Vaccine efficacy 80-100% against severe pertussis
- Antitoxin titers overlap
- Conclusion of no correlation between titer and protection
Varicella Example:
VZV Antibody Response 6 Weeks Postvaccination (1 Dose) by Varicella Breakthrough Status

Strong correlation between titers and protection based on statistical modeling of the whole titer distribution

Population-Based Correlates of Protection
Identification of Protective Level by Comparing Antibody Titers of Protected Group and Susceptibles
Population-Based Correlates of Protection (Siber, DBS 1997)

- Compare antibody levels in the protected group and the susceptible group
- Identify a threshold level achieved by most individuals in the protected group and not reached by most susceptibles
  - Estimate the minimum protective level
- Only require limited serological sampling (e.g., 10% cohort)

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**Haemophilus Influenzae b (Hib) Vaccine**

- Studies showed PRP polysaccharide vaccine has 88% efficacy in children >18 months of age and no benefit in younger children
- Käythy et al showed post-vaccination anti-PRP level of ≥1.0 µg/ml best discriminated between immunized and control populations aged 18 months or older.
  - This level indicates long-term protection
  - Accepted as criterion for licensing new Hib vaccines
Anti-PRP titer Responses

<table>
<thead>
<tr>
<th>Age</th>
<th>Anti-PRP ≥ 1 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immunized</td>
</tr>
<tr>
<td>6-11 mos.</td>
<td>16%</td>
</tr>
<tr>
<td>12-17 mos.</td>
<td>44%</td>
</tr>
<tr>
<td>18-23 mos.</td>
<td>75%*</td>
</tr>
<tr>
<td>24-36 mos.</td>
<td>90%*</td>
</tr>
</tbody>
</table>

* Protected population

Respiratory Syncytial Virus (RSV)

- RSV Lower respiratory infection (LRI) accessed during RSV immune globulin infusion
- LRI reduction of 63% in high-dose group and 27% in low-dose group relative to control
- Potential protective level is 200
Pertussis Vaccine Example

Swedish Acellular Pertussis Vaccine Trial in 1985-86

- Titers clearly separated between vaccine and placebo groups

(Siber, DBS 1997)

Concerns with Population-Based Method

- The definition of protection is somewhat arbitrary and not rigorous
- The level of protection changes abruptly as implied by the protective level
  - Perhaps more likely to vary as a continuous function of antibody level
Titer-Specific Correlates of Protection

Siber, DBS 1997; Jódar et al, Vaccine 2003; Siber et al, Vaccine 2007

Titer-Specific Method

- Model the risk of disease as a continuous function of antibody titer
  - Logistic regression is often used
  - Other models have also been used
  - A step function may be used to establish a protection level
- More rigorous than the population-based method
Titer-Specific Method

- Obtain antibody titers in all individuals who develop disease in vaccinated and control groups
- Obtain antibody distribution in the entire population
  - Can be estimated using a random sample of study population
- Calculate titer-specific rate of disease:

Titer-Specific Method

- Fit a statistical model to evaluate the relationship between titer and disease risk
- For example, a logistic regression:

  \[ X = \text{antibody titer level} \]

- A step function can be used to estimate the protective level
A Logistic Regression Model
(Siber et al 2007)

Pertussis Vaccine Example
Swedish Acellular Pertussis Vaccine Trial in 1985-86

- Titers >16 are associated with a substantially lower risk

(Siber, DBS 1997)
RSV Example

- RSV LRI risk decreases as antibody titer increases.
- Titer of ≥200 is associated with a relative risk of 0.17 (83% efficacy).

(Siber, DBS 1997)

Pneumococcal Vaccine Example

Developing Serological Criteria for Licensing New Vaccines
Background

A 7-valent pneumococcal vaccine (Prevnar®) was approved in 2000 for immunization of infants and toddlers for prevention of invasive pneumococcal disease (IPD) caused by *Streptococcus pneumoniae*

- IPD includes bacteremia (bloodstream infection) and meningitis (infection of the membranes surrounding the brain and spinal cord)
- The seven serotypes (strains) of *S. pneumoniae* included in the vaccine are 4, 6B, 9V, 14, 18C, 19F, and 23F
- Vaccine given as 4-dose series at 2, 4, 6 and 12-15 months of age

Efficacy of Prevnar®

Three placebo controlled efficacy trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Evaluable N</th>
<th>Case Split (Vaccine/Control)</th>
<th>Vaccine Efficacy (VE, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCKP*</td>
<td>21,935</td>
<td>1/39</td>
<td>97.4% (82.7, 99.9)</td>
</tr>
<tr>
<td>American Indian</td>
<td>5,792</td>
<td>2/8</td>
<td>76.8% (-9.4, 95.1)</td>
</tr>
<tr>
<td>South Africa</td>
<td>37,107</td>
<td>1/10</td>
<td>90.0% (29.7, 99.8)</td>
</tr>
</tbody>
</table>

*Northern California Kaiser Permanente
Need for Additional Vaccines

- Prevnar does not contain additional serotypes, such as 1 and 5, that are an important cause of IPD in South America, Africa, and Asia
- New vaccines containing 9, 11, 13 and 15 serotypes are being developed
- Prevnar 13™ was approved in US in 2010 based on immunogenicity bridging
  – Additional serotypes: 1, 3, 5, 6A, 7F, and 19A

Proposed Licensure Criteria for New Pneumococcal Vaccines

- Placebo-controlled trial not ethical
- Noninferiority of new vaccines to Prevnar must be established
  – Efficacy trial not feasible due to high efficacy of Prevnar
  – Immunogenicity trial is possible but clear serological criteria need to be established
- Discussion at FDA Advisory Committee in 2001
- World Health Organization (WHO) formed a working group in 2002 to recommend serological criteria that will predict protective efficacy
Serology Assay – IgG ELISA

- IgG ELISA measures the IgG antibody
- Shown to correlate with opsonophagocytic (functional) activity (OPA) measured by Opsonophagocytic assay
- Post dose-3 antibody response correlates with booster response
- ELISA assay has been validated and standardized across multiple laboratories
- Serology data available from efficacy trials to assess correlates of protection

Correlation between ELISA and OPA Titer

Jódar et al, Vaccine 2003
Correlation between Primary and Booster Responses

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>Pearson correlation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.441</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6B</td>
<td>0.526</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>9V</td>
<td>0.439</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>14</td>
<td>0.341</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18C</td>
<td>0.408</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>19F</td>
<td>0.306</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>23F</td>
<td>0.545</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>


*Correlation based on post dose 3 and post dose 4 Ab concentrations on log-scale.

Jódar et al, Vaccine 2003

Statistical Framework

- Assume a theoretical underlying logistic regression model between antibody tiers and risk of IPD
- Assume a step-function to define a specific protective level
- Assume the relationship is the same for all serotypes
  - Allowing pooling of efficacy and immunogenicity data
- Assume antibody at 4 weeks post dose 3 (primary series) is important in predicting long-term protection
The Logistic Regression Model
(Siber et al 2003)

Step Function Model
and Vaccine Efficacy (VE)

\[ [C]_{prot} = \text{protective level of antibody} \]
\[ p_v = \% \text{ subjects with antibody levels} < [C]_{prot} \]
\[ \text{in the vaccinated group} \]
\[ p_c = \% \text{ subjects with antibody levels} < [C]_{prot} \]
\[ \text{in the control group} \]
\[ a = \text{Prob of IPD when antibody} < [C]_{prot} \]
\[ b = \text{Prob of IPD when antibody} \geq [C]_{prot} \]
Step Function Model and Vaccine Efficacy (VE)

\[ P(\text{IPD in vaccines}) = a_p + b (1 - p_v) \]
\[ P(\text{IPD in controls}) = a_p + b (1 - p_c) \]

If the prob of IPD when antibody \( \geq [C]_{prot} \) is very small (\( b \) is close to zero):

Relative risk of IPD = Relative risk of having antibody titer < \( [C]_{prot} \)

When VE is known, \( [C]_{prot} \) may be determined directly from the reverse cumulative distribution curves (RCDC) of the antibody titers
- If antibody titers in placebo group is very low, it can be ignored in determining \( [C]_{prot} \)
Step Function Model and Vaccine Efficacy (VE)

- Variability in $[C]_{prot}$ estimate depends on the variability of VE estimate and serology data
  - Dominated by VE variability if serology sample size is large
  - CI for $[C]_{prot}$ determined using CI for VE

Determining Protective Level Based on RCDC

Jódar et al, Vaccine 2003
Estimated Protective Pneumococcal Antibody Level

<table>
<thead>
<tr>
<th>Study</th>
<th>Observed VE</th>
<th>Estimated $[C]_{prot}$ (µg/ml)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCKP</td>
<td>97.4%</td>
<td>0.20</td>
<td>(0.03, 0.67)</td>
</tr>
<tr>
<td>American Indian</td>
<td>76.8%</td>
<td>1.00</td>
<td>(0.25, &gt;50.0)</td>
</tr>
<tr>
<td>South Africa</td>
<td>90%</td>
<td>0.68</td>
<td>(0.03, 6.0)</td>
</tr>
<tr>
<td>Pooled* (weighted)</td>
<td>93%</td>
<td>0.35</td>
<td>(0.11, 0.85)</td>
</tr>
</tbody>
</table>

Weighted by the number of subjects in the trial
WHO Recommendations for Licensure of New Pneumococcal Vaccines

- Noninferiority study of immunogenicity is acceptable.
- The percent of subjects achieving an ELISA antibody titer by of $\geq 0.35 \, \mu g/ml$ is a useful benchmark (can be used as primary endpoint).
- More emphasis on assessing the OPA titers as they reflect the functionality of antibody, especially for new serotypes not included in the original vaccine.
  - Explore the relationship between ELISA and OPA.

Evaluating Protective level using Receiver Operating Characteristic Curve (ROC)
Evaluating Protective level using Receiver Operating Characteristic Curve (ROC)

- Consider disease status as gold standard and assay value as diagnostic test
  Test positive = assay value < protective level
- Evaluate the sensitivity and specificity of the test for a variety of cutoff value
- Choose the optimal cutoff value to be the protective level based on ROC
  – Balance sensitivity and specificity

Sensitivity and Specificity

Sensitivity = TP/(TP+FN)  Specificity = TN/(FP+TN)

<table>
<thead>
<tr>
<th>Test (Y)</th>
<th>Disease Status (Z)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Positive</td>
<td>True + (TP)</td>
<td>False + (FP)</td>
</tr>
<tr>
<td>Negative</td>
<td>False – (FN)</td>
<td>True – (TN)</td>
</tr>
<tr>
<td>Total</td>
<td>TP + FN</td>
<td>FP + TN</td>
</tr>
</tbody>
</table>
Balance Between Sensitivity and Specificity

Receiver Operating Characteristic Curve
- Choose the cutoff value that has high sensitivity and specificity
Further thoughts on ROC Methods
(Li, Parnes and Chan, JBS 2013)

Identify the cutoff threshold

- By maximizing the correlation between Disease (Z) and immune responses (Y)
- By minimizing the misclassification rate:
  \[ MR = \pi \times fn + (1 - \pi) \times fp \]

\( \pi \) is the disease prevalence