

ISCB2010
Special Day Vaccines

ANCOVA of Antibody Titers
and
a Solution to the Problem of
Heteroscedasticity

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**This presentation represents
my personal view**

Antibodies

After infection the immune system produces antibodies against (antigens of) the invading pathogen

Antibodies mark the invaders for destruction, or inactivate them

For many infectious diseases antibodies offer life-long protection

Vaccination

Vaccination = mimicking infection
(but with inactivated pathogen)

Immune system reacts by producing
antibodies

When exposed to natural pathogen
these antibodies prevent infection

Protection

Most vaccines offer life-long protection, e.g. most childhood vaccines

Some vaccines do not offer life-long protection, e.g. tetanus vaccine

Antibody Titers

Antibody levels measured as titers

Blood sample is serially diluted, e.g.
1:10, 1:20, 1:40, 1:80, etc.

Titer = reciprocal highest dilution still
giving certain assay read-out, e.g. 40

Antibody Titters

Log transformed antibody titters
usually Normally distributed

Pre-vaccination Antibody Levels

Pre-vaccination antibody levels are often below detection level of assay
(assigned log titer: 0)

But in case of repeated vaccination
this need not be the case

Imbalance in Baseline Antibody Titers

In comparative studies imbalance in baseline antibody titers can occur

How to correct for this imbalance?

Standard approaches

Change-from-baseline analysis

Stratification

Analysis of Covariance

Change-from-baseline analysis

Fold increase = post-vaccination titer
/ pre-vaccination titer

log transformed fold increase is a
change score

Does log fold increase analysis
eliminate baseline imbalance?

Change-from-baseline analysis

It is a fallacy that change-from-baseline analysis eliminates baseline imbalance

If pre- and post-treatment values positively correlated, then pre- and change scores also correlated, albeit negatively

Pre-Vaccination Stratification

Stratification based on baseline titer
difficult because blood samples must
be assayed (extra visit needed)

Not possible in case of combination
vaccine

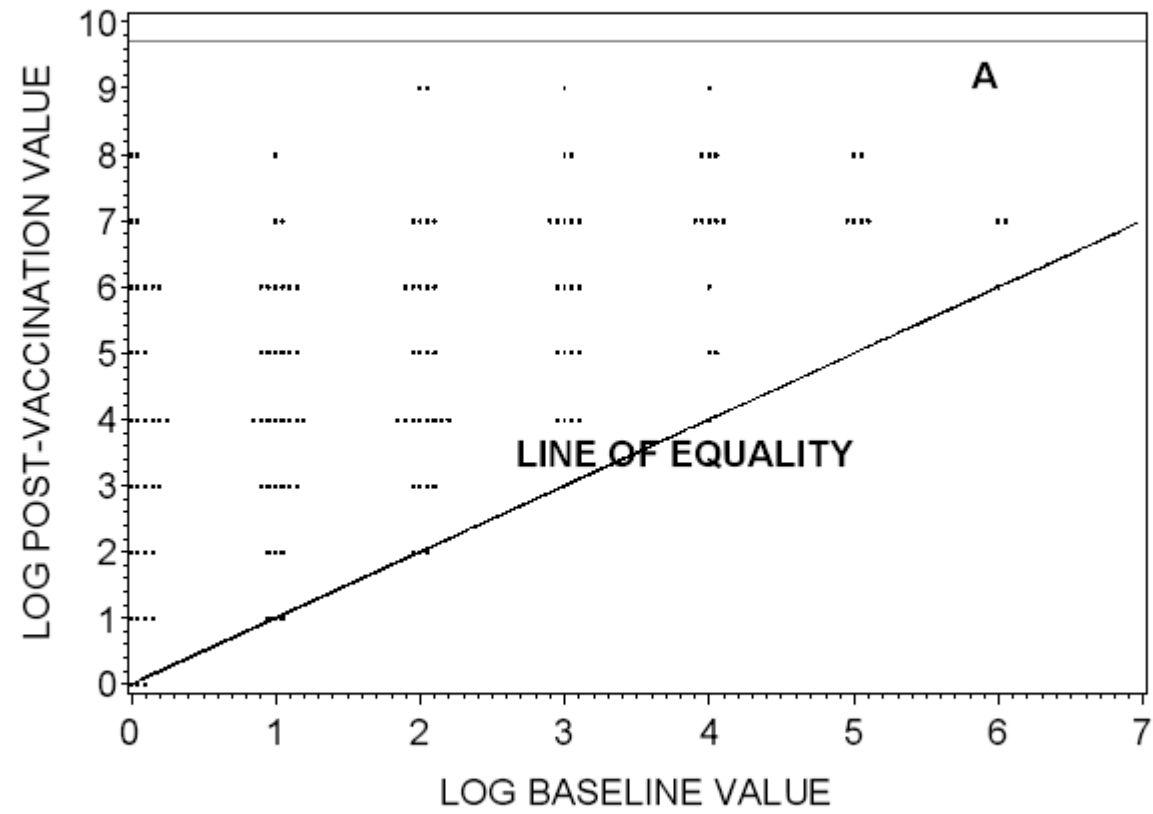
Post-Vaccination Stratification

Stratification during statistical analysis is a possibility

Analysis of Covariance

Limiting case of stratification

Preferred because most efficient approach (see e.g. Senn *Statistical Issues in Drug Development*)



ANCOVA

Simplest case: single group

$$Y = \alpha + \beta.z + E_z$$

Y: log post-vaccination titer

z: log pre-vaccination titer

Assumption of Homoscedasticity

Error term \mathbf{E}_z Normally distributed
with mean μ_z and standard dev. σ_z

and

$$\sigma_z = \sigma$$

(homoscedasticity)

ANCOVA Antibody Titters: Heteroscedasticity

Assumption of homoscedasticity
does not hold

σ_z decreases with increasing z ,
see Figure 1

(example of heteroscedasticity)

ANCOVA antibody titers

If heteroscedasticity is ignored,
estimates, confidence intervals
and P-values will be invalid

Heteroscedasticity: Solution

Model the variance

Variance model

For Normal data crude formula for range of values to be observed:

range $\approx c \times$ standard deviation

c a constant (often: 4 or 6)

Variance model

Range log transformed
post-vaccination titers (A-z)

A asymptote in Figure 1

$$(A-z) \approx c\sigma_z$$

Variance model for E_x

$$\sigma_z^2 \approx \sigma^2(1 + c_1 z + c_2 z^2)$$

$$\sigma^2 = (A/c)^2$$

$$c_1 = -2/A$$

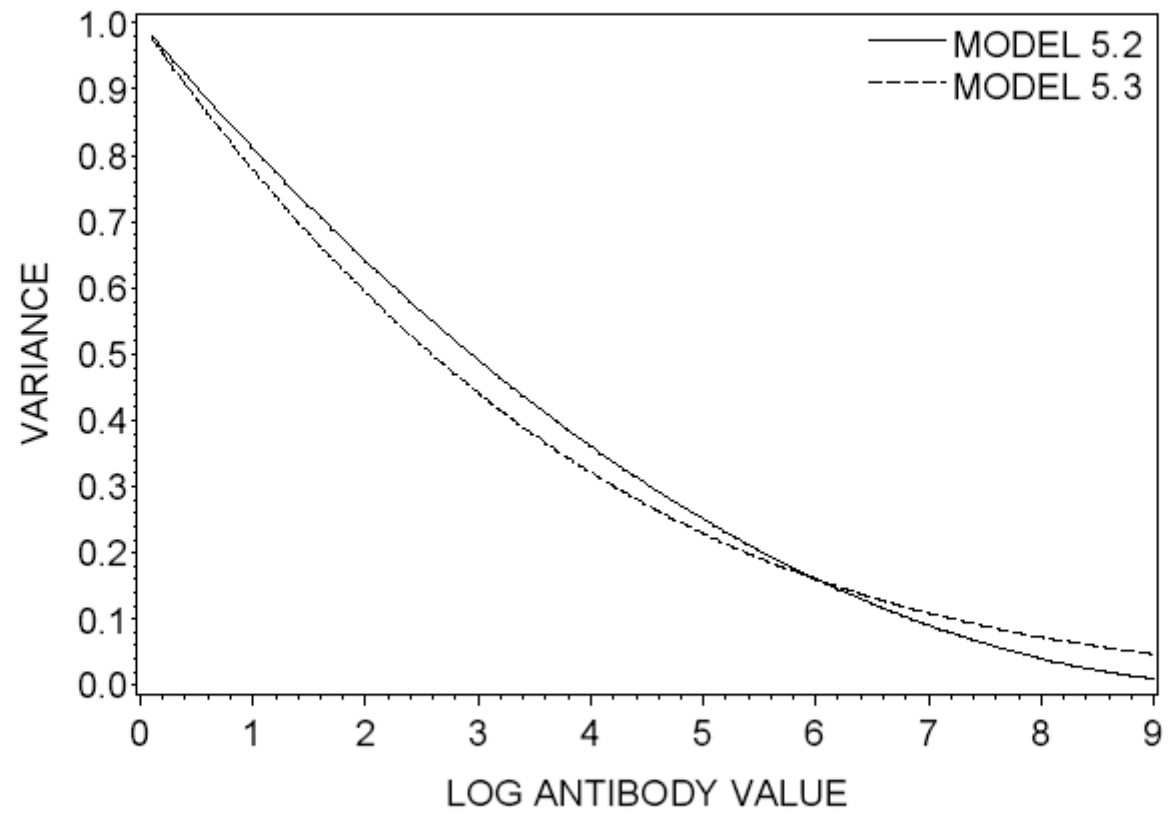
$$c_2 = 1/A^2$$

Fitting the variance model

Variance model cannot be fitted
with SAS

But an almost similar model can:

$$\sigma_z^2 \approx \sigma^2 \exp(C_1 z + C_2 z^2)$$



Fitting the model with SAS

$z^2 = z * z;$

```
proc mixed;  
model y=z / solution;  
repeated / local=exp(z z2);  
run;
```

Fitting the model with SAS

Estimates for α , β , σ and C_1 , C_2
are returned

C_1 and C_2 are
nuisance parameters

Seronegative subjects

Seronegative subjects: $z=0$

α and σ can also be estimated
from data of seronegative
subjects (subset)

Fitting model to data Figure 1

homoscedasticity:

$n=163$; $\alpha=3.71$; $\beta=0.70$; $\sigma=1.87$

heteroscedasticity:

$n=163$; $\alpha=3.76$; $\beta=0.64$; $\sigma=2.15$

seronegative subjects:

$n=55$; $\alpha=3.82$; $\sigma=2.21$

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Statistics in Clinical Vaccine Trials

 Springer