STUDY DESIGNS

IN BIOMEDICAL RESEARCH

FIELLER'S THEOREM FOR

THE ESTIMATION OF RATIO OF PARAMETERS

THE GAP

- Most teaching and learning programs in Statistics and Biostatistics focus on the differences and the sums of parameters, statistics, or random variables.
- However, in many applications we have to deal with ratios of parameters, statistics, or random variables.
- That is, Statistics puts more emphasis on “additive models”; most plausible biological and biomedical models are “multiplicative”.
- The following are a few examples:

RELATIVE RISK

- *Relative Risk* has been a popular parameter in epidemiology studies; a concept used for the comparison of two groups or populations with respect to an unwanted event.
- It is the ratio of incidence rates or disease prevalences; usually, one group is under standard condition against which the other group (exposed group) is measured.
- Relative Risk is a ratio: Risk Ratio, it is a ratio of two proportions.

ODDS RATIO

- When incidence and prevalence are low (rare diseases), the *Relative Risk* and the *Odds Ratio* are approximately equal.
- Odds Ratio is more popular because it is estimable in retrospective designs; in practice, we calculate Odds Ratio and interpret it like Relative Risk.
- But Odds Ratio is still a ratio of parameters; maybe it's a different kind of ratios – a ratio of ratios

DIAGNOSTIC TESTS

- Some of the indices of diagnostic accuracy are the "Likelihood Ratios", each is the ratio of two probabilities
- Both are expressible as functions of sensitivity and specificity.

$$LR^{+} = \frac{\Pr(T = + \mid D = +)}{\Pr(T = + \mid D = -)} = \frac{S^{+}}{1 - S^{-}}$$

$$LR^{-} = \frac{\Pr(T = - \mid D = +)}{\Pr(T = - \mid D = -)} = \frac{1 - S^{+}}{S^{-}}$$

COMPARISON OF SCREENING TESTS WITH BINARY ENDPOINT

We can perform two separate Chi-square tests or McNemar Chi-square tests – depending on the design, one for cases and one for controls; for an overall level of α, each test is performed at $\alpha/2$. That is, we compare sensitivities and we compare specificities separately: No Problem here.

MEASURING DIFFERENCES

- If the difference between two diagnostic tests are found to be significant; the level of difference should be summarized and presented.
- The two commonly used parameters are the ratio of two sensitivities (RS^+) and the ratio of two specificities (RS^-); ratios of two proportions.

DIRECT ASSAYS

- In direct assays, the doses of the standard and test preparations are “directly measured” for an “event of interest” (with intra-subject dose escalation).
- When an event of interest occurs, e.g.. the death of the subject, and the variable of interest is the dose required to produce that event for each subject. The value is called “individual effect dose” (IED).
- We have 2 independent samples

Since the “concentration” and the “dose” are inversely proportional - when concentration is high, we need a smaller dose to reach the same response. In other words , we define the “relative potency” or “ratio of concentrations” of the test to standard as the “ratio of doses” of the standard to test:

$$\rho = \frac{Dose_S}{Dose_T} = \frac{\mu_S}{\mu_T}$$

That is a "Ratio of Means"

QUANTITATIVE ASSAYS

- A common approach for parallel line assays and slope ration assays is pooling data from both preparations and using “Multiple Regression”.
- Dependent Variable: Y = Response;
  Two Independent Variables are: X = Dose (Slope ratio) or log(Dose) (Parallel line) & P = Preparation (a “dummy variable” coded as P = 1 for “Test” and P = 0 for “Standard”)

PARALLEL-LINE ASSAYS

Multiple Regression Model :

$$E(Y) = \beta_0 + \beta_1 X + \beta_2 P$$

β_1 is the common slope and

β_2 is the "difference of intercepts";

$$M = \log \rho = \frac{\beta_2}{\beta_1}$$

That is "**Ratio of Regression Coefficients**"

SLOPE-RATIO ASSAYS

Multiple Regression Model #1 :

$$E(Y) = \beta_0 + \beta_1 X + \beta_2 PX$$

β_0 is the common intercept and

$$\beta_T = \beta_1 + \beta_2$$

$$\beta_S = \beta_1$$

$$\rho = \frac{\beta_1 + \beta_2}{\beta_1} = 1 + \frac{\boldsymbol{\beta_2}}{\boldsymbol{\beta_1}}$$

That involves a "**Ratio of Regression Coefficients**"

QUANTAL ASSAYS

Quantal Assays and Dose-ranging Experiments are modeled by Logistic Regression with drug used on the log scale (implied by the Median Effects Principle). Setting the proportion of response equal to 0.5, we can determine ED50, a measure of potency; it is a function of the ratio of slope to intercept:

$$\pi = \frac{e^{\beta_0 + \beta_1 x}}{1 + e^{\beta_0 + \beta_1 x}}$$

$$1 - \pi = \frac{1}{1 + e^{\beta_0 + \beta_1 x}}$$

$$\frac{\pi}{1 - \pi} = e^{\beta_0 + \beta_1 x}$$

$$\log \frac{\pi}{1 - \pi} = \beta_0 + \beta_1 x$$

How do we estimate the ratio of two parameters, e.g. ratio of two population means in Direct Bioassays? Numerator and denominator can be estimated by method such as Maximum Likelihood (MLE).

POINT ESTIMATE

$$r = \frac{A}{B}$$

Both statistics, A and B, are asymptotically distributed as "**normal**" with "estimable variances"

$$\mathbf{r} = \frac{\mathbf{A}}{\mathbf{B}}$$

A and B could be **sample means** (direct assays), **proportions** (relative risk), or **regression coefficients** (quantitative assays)

What about Confidence Intervals, such as 95% Confidence Interval?

First, we need Standard Error of the point estimate. For example, ne can assume that r is normally distributed, obtain the variance and standard error by the Delta's method (also called error propagation), then form confidence intervals for ρ the usual way (r is an estimate of ρ).

There are two problems here:

(1) Delta method provides only an approximation (this maybe a minor problem);

(2) The ratio of two normal variates is not normally distributed; this could be serious.

$$\log r = \log A - \log B$$

The more popular alternative is taking logs; in forming confidence intervals for ρ (r is an estimate of ρ), we obtain the variance and standard error of log(r) by the Delta's method, form confidence intervals for log(ρ) the usual way. Then exponentiating the endpoints of the confidence interval for log(ρ) to obtain a confidence interval for ρ.

In doing so, we assume that log(A) and log(B) are normally distributed which contradict the fact that A and B themselves are normally distributed. The result is based on inflated variances (variance of lognormal distribution is larger than variance of normal distribution) which is inefficient because confidence intervals are too long – unnecessarily.

Example: Focusing on Risk Ratio (ratio of 2 proportions, Lui (Contemporary Clinical Trials, 2006) found that the log transformation method could lead to intervals which are many times longer than those by competing methods - as much as 40 times in some configurations – an obvious loss of "efficiency".

This lecture covers a method, called Fieller's Theorem, aiming to fill this gap. Fieller's Theorem is an efficient statistical method which directly provides confidence intervals for ratios of two parameters – without calculating standard errors:

Fieller, E.C. 1944. "A Fundamental Formula in the Statistics of Biological Assay, and Some Applications," *Quarterly Journal of Pharmacy and Pharmacology*, 17: 117-123.

FIELLER'S THEOREM

If r = A/B is an estimate of ρ, we consider the statistic (A- ρB) which is distributed as normal because both A and B are normally distributed and is ρ a constant. We derive mean and variance of that statistics which lead to confidence limits for ρ.

Let C = A- ρB, distributed as normal
We first find the mean & variance of C

Recall: C = A- ρB is distributed as normal
We first find the mean & variance of C

$$E(C) = 0$$

$$\mathrm{Var}(C) = V;\ V \text{ is estimated by } v$$

$$C/\sqrt{v} \text{ is distributed as "t"}$$

$$\Pr(-t_{.975} \le C/\sqrt{v} \le t_{.975}) = .95;$$

$$\Pr(C^2/v \le t^2_{.975}) = .95$$

$$\Pr(C^2 \le v t^2_{.975}) = .95$$

$$\Pr(C^2 \le vt^2_{.975}) = .95$$

$$\Pr\{(A - \rho B)^2 \le vt^2_{.975}) = .95;$$

Solve the "quadratic equation":

$$\mathbf{(A - \rho B)^2 = vt^2_{.975}}$$

to obtain lower and upper limits for ρ

The two solutions (or roots of that quadratic equation) are the lower and the upper endpoint of the 95% Confidence Interval for the (unknown) ratio ρ.

DIRECT ASSAYS: RATIO OF MEANS

$$E(\overline{X_S} - \rho\overline{X_T}) = 0$$

$$Var(\overline{X_S} - \rho\overline{X_T}) = \sigma^2\left(\frac{1}{n_S} + \frac{\rho^2}{n_T}\right)$$

$$\Pr[\{\overline{X_S} - \rho\overline{X_T}\}^2 \le t^2_{.975}s^2_p\left(\frac{1}{n_S} + \frac{r^2}{n_T}\right)] = .95$$

where $t_{.975}$ is the 97.5th percentile of the t distribution with (n_S + n_T - 2) degrees of freedom.

The two roots obtained by solving the quadratic equation within the probability statement yielding the 95% confidence limits r_L and r_U.

Recall:

When you have a quadratic equation $ax^2 + bx + c = 0$; first step is checking b^2-4ac. If it's positive, 2 roots exist:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

$$\{\overline{X_S} - \rho\overline{X_T}\}^2 = t_{.975}^2 s_p^2 (\frac{1}{n_S} + \frac{r^2}{n_T})$$

$$\overline{X_T}^2 \rho^2 - 2\overline{X_S}\,\overline{X_T}\rho + \{\overline{X_S}^2 - t_{.975}^2 s_p^2 (\frac{1}{n_S} + \frac{r^2}{n_T})\} = 0$$

Solve for ρ : two roots exist because

$$\{-2\overline{X_S}\,\overline{X_T}\}^2 - 4\overline{X_T}^2 \{\overline{X_S}^2 - t_{.975}^2 s_p^2 (\frac{1}{n_S} + \frac{r^2}{n_T})\} > 0$$

Suggested Exercise:

Try to fill in the details for the results in the following slides

RESULTS FOR RELATIVE POTENCY

The first one is the 95% CI directly from the Fieller's theorem, the second one is an approximation because the term "g" is often rather small.

$$\frac{1}{(1-g)}\left\{r \pm t_{.975}\frac{s_p}{x_T}\sqrt{\frac{1}{n_S}(1-g)+\frac{r^2}{n_T}}\right\}$$

$$g = \frac{t_{.975}^2 s_p^2}{n_T \bar{x}_T^2}$$

$$r \pm t_{.975}\frac{s_p}{x_T}\sqrt{\frac{1}{n_S}+\frac{r^2}{n_T}}$$

"Exact"

Approximation

The approximation is in more standard form: point estimate plus/minus margin of error.

EXAMPLE

	Standard	Test
	2.42	1.55
	1.85	1.58
	2	1.71
	2.27	1.44
	1.7	1.24
	1.47	1.89
	2.2	2.34
Total	13.91	11.75
Mean	1.987	1.679
Variance	0.1136	0.1265

$$\frac{1}{(1-g)}\left\{r \pm t_{.975}\frac{s_p}{\bar{x}_T}\sqrt{\frac{1}{n_S}(1-g)+\frac{r^2}{n_T}}\right\}$$

$$g = \frac{t^2_{.975}s^2_p}{n_T\bar{x}^2_T}$$

$$t_{.975}(12df) = 2.179$$

$$s_p = \sqrt{\frac{(6)(.1136)+(6)(.1265)}{12}} = .3464$$

$$g = \frac{1}{7}\left\{\frac{(2.179)(.3464)}{1.679}\right\}^2 = .029$$

$$\frac{1}{1-.029}\left\{1.18 \pm (2.179)\frac{.3464}{1.679}\sqrt{\frac{1-.029}{7}+\frac{(1.18)^2}{7}}\right\}$$

$$= (0.95, 1.48)$$

APPROXIMATE RESULT

	Standard	Test
	2.42	1.55
	1.85	1.58
	2	1.71
	2.27	1.44
	1.7	1.24
	1.47	1.89
	2.2	2.34
Total	13.91	11.75
Mean	1.987	1.679
Variance	0.1136	0.1265

$$g = \frac{t_{.975}^2 s_p^2}{n_T \bar{x}_T^2}$$

$$r \pm t_{.975} \frac{s_p}{\bar{x}_T} \sqrt{\frac{1}{n_S} + \frac{r^2}{n_T}}$$

$$t_{.975}(12df) = 2.179$$

$$s_p = \sqrt{\frac{(6)(.1136)+(6)(.1265)}{12}} = .3464$$

$$1.18 \pm (2.179)\frac{.3464}{1.679}\sqrt{\frac{1}{7}+\frac{(1.18)^2}{7}}\}$$

$$= (0.92, 1.44)$$

vs. (.95,1.48)

EPIDEMIOLOGY: RELATIVE RISK

$$\rho = \frac{\pi_2}{\pi_1}$$

$$r = \frac{p_2}{p_1}$$

$$C = p_2 - \rho p_1$$

$$Var(C) = \frac{\pi_2(1-\pi_2)}{n_2} + \rho^2 \frac{\pi_1(1-\pi_1)}{n_1}$$

$$C = p_2 - \rho p_1$$

$$Var(C) = \frac{\pi_2(1-\pi_2)}{n_2} + \rho^2 \frac{\pi_1(1-\pi_1)}{n_1}$$

$$\hat{Var}(C) = \frac{p_2(1-p_2)}{n_2} + (\frac{p_2}{p_1})^2 \frac{p_1(1-p_1)}{n_1}$$

$$\frac{(p_2 - \rho p_1)^2}{\hat{Var}(C)} = z^2_{1-\alpha/2}$$

Two roots form $(1-\alpha)100\%$ C. I. for ρ

(Similar to approach in ratio of means: we use estimated variance in last step)

Back to the introduction of Fieller's Theorem; noted that we estimate the variance of C (which is a function of the ratio to be estimated) before forming the "t" statistic. The alternative is using the variance of C – instead of its estimate (This would provide "exact" result/solution).

FIELLER'S THEOREM

If r = A/B is an estimate of ρ, we consider the statistic (A- ρB) which is distributed as normal because both A and B are normally distributed and is ρ a constant. We derive mean and variance of that statistics which lead to confidence limits for ρ.

Let C = A- ρB, distributed as normal
We first find the mean & variance of C

Recall: C = A- ρB is distributed as normal
We first find the mean & variance of C

$$E(C) = 0$$

$$\mathrm{Var}(C) = V;\ V \text{ is estimated by } v$$

$$C/\sqrt{v} \text{ is distributed as "t"}$$

$$\Pr(-t_{.975} \le C/\sqrt{v} \le t_{.975}) = .95;$$

$$\Pr(C^2/v \le t^2_{.975}) = .95$$

$$\Pr(C^2 \le v t^2_{.975}) = .95$$

$$\Pr(C^2 \le vt^2_{.975}) = .95$$

$$\Pr\{(A - \rho B)^2 \le vt^2_{.975}) = .95;$$

Solve the "quadratic equation":

$$\mathbf{(A - \rho B)^2 = vt^2_{.975}}$$

to obtain lower and upper limits for ρ

$$\Pr(C^2 \le Vt^2_{.975}) = .95$$

$$\Pr\{(A - \rho B)^2 \le Vt^2_{.975}) = .95;$$

Solve the "quadratic equation":

$$\mathbf{(A - \rho B)^2 = Vt^2_{.975}}$$

to obtain lower and upper limits for ρ

If we use the variance of C instead of its estimate, the parameter is involved on both sides of the last equation. The quadratic equation becomes more complicated but we would get the real "exact" result for the confidence interval.

EXAMPLE: RELATIVE RISK

$$C = p_2 - \rho p_1$$

$$Var(C) = \frac{\pi_2(1-\pi_2)}{n_2} + \rho^2 \frac{\pi_1(1-\pi_1)}{n_1}$$

$$\frac{(p_2 - \rho p_1)^2}{Var(C)} = z^2_{1-\alpha/2}$$

Two roots form $(1-\alpha)100\%$ C. I. for ρ

(Liu, Contemporary Clinical Trials 2006)

The real exact result and the approximated result (by first estimating the variance of C) are often very close. Liu (CCT, 2006) used variance and obtained exact result; he got into a new problem: the resulting quadratic equation may have no real roots in some simulation configurations.

Lui (Contemporary Clinical Trials, 2006) applied Fieller's Theorem to study "Risk Ratio"; showed that the use of Fieller's Theorem/method would lead to more efficiency (i.e. shorter intervals) but, more important, it improves coverage probability. I believe that the results apply to quantitative and quantal bioassays– e.g. ratio of regression coefficients . It was confirmed in a Plan B (2018) for the case of Dose-ranging Experiments.

The cases of ratio of means (in Direct Bioassays) and ratio of proportions (Relative Risk in Epidemiology) are more simple; A (numerator) and B(denominator) come from independent samples, so they are not correlated. For the case of ratio of regression coefficients (for example, in Indirect Bioassays), we have to include the covariance of A and B.

RATIO OF REGRESSION COEFFICIENTS

$C = A - \rho B$

$V = Var(C)$

$= Var(A) + \rho^2 Var(B) + 2\rho Cov(A, B)$, estimated by

v = $Var(A) + (\frac{A}{B})^2 Var(B) + 2(\frac{A}{B}) Cov(A, B)$

The estimating equation becomes

$$0.95 = P[C^2 \leq v Z^2_{0.975}]$$

$$B^2\rho^2 + 2AB\rho + A^2 - [Var(A) + (\frac{A}{B})^2 Var(B) + 2(\frac{A}{B}) Cov(A, B)]\, z^2_{0.975} = 0$$

ODDS RATIO

- Does Fieller's Theorem work for Odds Ratio?
- Odds Ratio is a "ratio of ratios"; its estimated numerator and denominator are not normally distributed – more like log normal; is Fieller's Theorem-based method robust in this case?
- Maybe not, I do not know; at least I'm not sure.
- Perhaps the "log transformation" method works well for Odds Ratio; and it has been one of a few ratios that we handle properly.

Suggested Readings:

Search, find (and read) the article by Lui in Contemporary Clinical Trials, 2006.

Suggested Exercise:

Given data from the following parallel-line bioassay; use Fieller's Theorem to calculate a 95% confidence interval for the Relative Potency.

	Preparation					
	Standard Preparation			Test Preparation		
Dose (D; mmgcc)	0.25	0.50	1.00	0.25	0.50	1.00
X = log10(Dose)	-0.602	-0.301	0.000	-0.602	-0.301	0.000
Response (Y; mm	4.9	8.2	11.0	6.0	9.4	12.8
	4.8	8.1	11.5	6.8	8.8	13.6
	4.9	8.1	11.4	6.2	9.4	13.4
	4.8	8.2	11.8	6.6	9.6	13.8
	5.3	7.6	11.8	6.4	9.8	12.8
	5.1	8.3	11.4	6.0	9.2	14.0
	4.9	8.2	11.7	6.9	10.8	13.2
	4.7	8.1	11.4	6.3	10.6	12.8