

Biost 517

Applied Biostatistics I

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Lecture 16: Two Sample Inference for Correlated Response Data

November 27, 2006

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Lecture Outline

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- Dependent Data Within Clusters
- Matched Continuous Data
 - Paired t Test (means, geometric means)
 - Sign Test (median difference)
 - (Wilcoxon) Signed Rank Test
- Comparing Proportions: Matched Samples

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Dependent Data Within Clusters

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Dependent Data

.....

- There are times when data can not be presumed to be totally independent
 - Sampling within families
 - Sampling within schools, hospitals
 - Repeated measurements on individuals taken at a single time
 - Longitudinal data: repeated measurements taken on individuals over time

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Motivation for Longitudinal Data

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- Three settings in which longitudinal studies are performed
 - Convenience of existing study population
 - Efficiency of using subjects as own comparison
 - Scientific questions about effects that occur
 - over time, or
 - within subjects

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Convenience

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- Questions are truly cross-sectional
 - Multiple measurements made on each individual is easier than gathering new subjects
 - Natural variation within individuals provides additional information
 - E.g., Serum osmolality from Na, Glc, BUN
 - Interest is relationships between concurrent measurements

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Efficiency

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- Questions could be answered with cross-sectional study
- Primary comparison within subjects may have less variability
 - Allow detection of smaller effects
 - E.g., Adjusting for baseline measurements
 - E.g., Cross-over study of a new treatment

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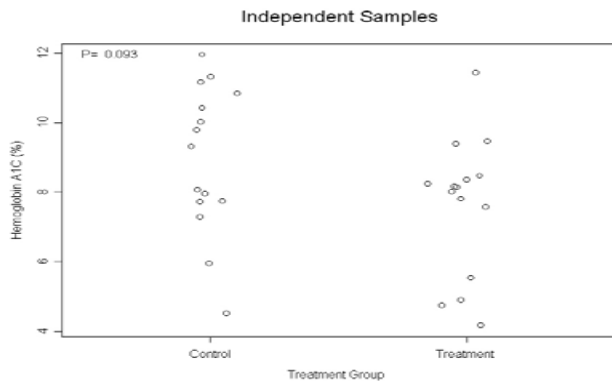
Example

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- Percent glycosylated hemoglobin is used to monitor long term control in diabetes
 - Hemoglobin A1c
- Consider studies of two insulin delivery strategies
 - Independent groups
 - Cross-over design

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Graph: Independent Samples



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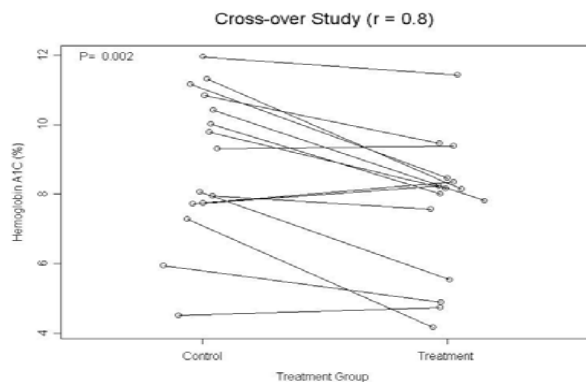
Inference: Independent Groups

- Large between-subject variability hampers our ability to detect differences
 - Between group SE is square root of sum of squared within group SEs
 - Within group SEs are proportional to within group standard deviation divided by the square root of n

$$se(\bar{X} - \bar{Y}) = \sqrt{\frac{\sigma_X^2}{n_X} + \frac{\sigma_Y^2}{n_Y}}$$

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Graph: Cross-over Study



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Inference: Cross-over Study

- High correlation between measurements taken on the same individual increases precision
 - The “random effect” of patient ID can be thought of as a precision variable

$$se(\bar{X} - \bar{Y}) = se(\bar{D}) = \sqrt{\frac{\sigma_X^2 + \sigma_Y^2 - 2\rho\sigma_X\sigma_Y}{n}}$$

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Longitudinal Questions

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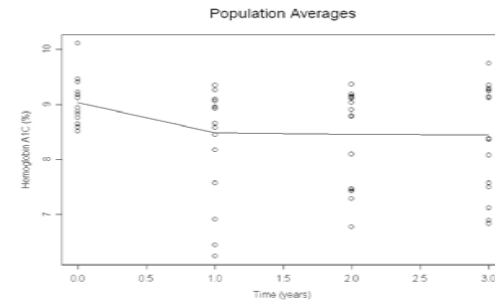
- Scientific questions about effects that occur over time
 - Studies to detect population time trends in response
 - E.g., rate (slope) of progression of retinopathy in population of diabetics over time
 - E.g., time to development of albuminuria

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Example: “Marginal Effects”

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- Time trends in group mean HbA1C
 - Note trends in mean and variability



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Within Subject Effects

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- Trends in specific individuals might not look like trends in population means
 - Response over time may be restricted to subgroups of subjects
 - Response over time may be transient

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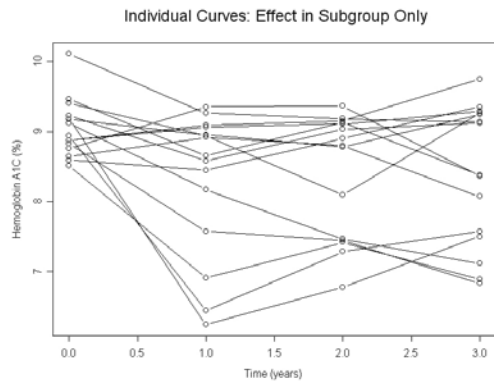
Longitudinal Scientific Questions

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- Scientific questions about effects that occur within subjects
 - Studies to detect time trends or covariate effects in individual response
 - E.g., distribution of rates (slopes) of progression of retinopathy in population over time
 - E.g., effect of varying risk factors within individuals

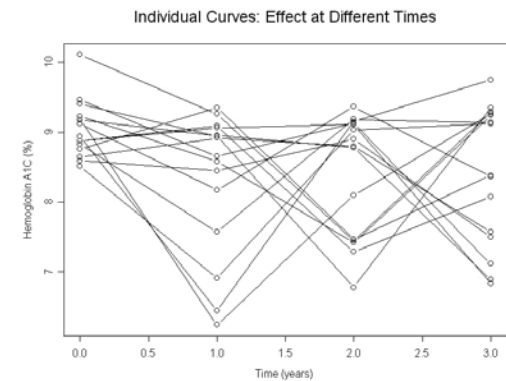
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Effect in Subgroup



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Transient Effects



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Choice of Measures of Outcome

- In order of importance
 - Scientific relevance
 - Including state of current knowledge
 - Plausibility of difference across groups
 - Statistical precision for analysis

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Longitudinal Outcome Measures

- In longitudinal studies, each individual may have multiple measurements over time
 - Definition of individual response thus can be based on multiple measurements
 - Response at a fixed time
 - Responses at multiple fixed times
 - Average response over time (area under curve)
 - Rate of change in response (slope)
 - Time to attaining some level of response

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Measures of Outcome

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- “Marginal” or population effects
 - Difference or ratio of group means, geometric means, medians, proportion or odds above threshold, hazards
 - Pr ($Y > X$)
- “Within subject” effects
 - Mean, median difference
 - Mean, geometric mean, median ratio
 - Within subject odds ratio
 - Pr ($Y > X$)

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Choice of Longitudinal Outcome

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- Should reflect scientific relevance, plausibility of effect, precision
 - Final level of response may be more important than earlier effects
 - (But in the long run, we are all dead)
 - Summarizing response at multiple time points reflects population rather than individuals
 - Average response over time sensitive to transient effects
 - Differences in time to event may be clinically meaningless

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Statistical Issues

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- Repeated measurements on subjects require special analysis techniques
 - May have erroneous conclusion if fail to account for correlated observations
 - Point estimates may be biased for population parameters
 - Too much emphasis placed on some subjects
 - Confidence intervals will not be accurate representation of our true confidence
 - P values will be wrong

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Statistical Approaches

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- Three basic approaches to analyzing correlated data
 - Reduce measurements on each cluster to a single observation; analyze across clusters
 - Estimate correlation within clusters and adjust standard errors for population based models
 - GEE, marginal models
 - “Robust” variance estimates
 - Adjust estimates for “random effects”
 - “Mixed effects models”: both fixed and random

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Easiest Approach

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- Reduce data for each individual to a single measurement
 - E.g., response at end of study, average response, rate of change
 - Analyses can then be based on standard methods for independent data
 - But:
 - Does not allow time-varying covariates
 - May not be most efficient statistically

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Example: Beta-carotene Data

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- Randomized clinical trial of beta-carotene supplementation on plasma levels of beta-carotene and vitamin E
 - Subjects randomized to 5 dose groups
 - Measurements at baseline, after 3 and 9 months of treatment, and 3 months after stopping treatment
 - Scientific question: How do plasma beta-carotene levels change over time within dose groups?
 - (effect modification between dose and time)

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Example: Beta-carotene Data

.....

- Reduce data to a single measurement on each subject
 - Difference between follow-up and baseline
 - Consider average of differences
 - No change corresponds to a difference of 0
 - Ratio between follow-up and baseline
 - Consider average of ratios
 - No change corresponds to a ratio of 1

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Example: SEP data

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- Somatosensory evoked potential measurements on healthy adults
 - Measurements of nerve conduction time
 - Four separate peaks for each leg of each subject
 - Reduce data to a single measurement
 - Consider only one peak on one leg
 - Which one?
 - Average measurements across peaks, legs
 - But will only generalize to similar averages
 - (Differences between peaks?)

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Matched Continuous Data

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Comparing Means

.....

- Paired t test
 - Compute differences for each pair
 - One sample t test that mean difference is 0
- Note that mean difference is difference of means
 - Same answer for population (“marginal”) and within subject questions (providing they both make sense)
 - May be inherent confounding, effect modification
 - E.g., age vs time vs birth year cohort effects

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Comparing Geometric Means

.....

- Paired t test on log transformed data
 - Compute differences for each pair
 - One sample t test that mean difference is 0
 - Back transform to consider geometric mean of ratios
 - Also geometric mean of ratios

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Sign Test

.....

- A very simple alternative test to the paired t test (which compares means) is to test whether the median of the differences is zero
 - If the median of the differences is zero, we would expect as many differences to be above zero as below zero
 - The differences that are exactly zero do not contribute much information about which measurement tends to be higher

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Median Difference

.....

- Compute differences of observations
 - Consider whether differences tend to be negative or positive

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Median Difference Properties

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- Median difference is not difference in medians
 - Ex: $X = (1, 3, 10)$; $Y = (2, 5, 10)$
 - $\text{mdn}(Y) - \text{mdn}(X) = 5 - 3 = 2$
 - Difference: $D = X - Y = (1, 2, 0)$; $\text{mdn}(D) = 1$
- The median difference is not transitive
 - Ex: $X = (1, 2, 3)$; $Y = (2, 3, 1)$; $Z = (3, 0, 2)$
 - $\text{mdn}(Y - X) = 1 > 0$ (so “Y larger than X”)
 - $\text{mdn}(Z - Y) = 1 > 0$ (so “Z larger than Y”)
 - $\text{mdn}(X - Z) = 1 > 0$ (so “X larger than Z”)

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Sign Test (Elevator Statistics)

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- Proportion positive among nonzero differences

$$X_i \stackrel{iid}{\sim} (\mu, \sigma^2) \quad Y_i \stackrel{iid}{\sim} (\nu, \tau^2) \quad D_i = X_i - Y_i \stackrel{iid}{\sim} (\mu - \nu, \omega^2)$$

P = number of D_i 's > 0

N = number of D_i 's < 0

If the median difference is 0, the number of positive differences is binomially distributed:

$$H_0 : P \sim B(P + N, 0.5)$$

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Sign Test: Stata Commands

.....

- Stata has a command to perform the sign test
 - “`signtest var1 = var2`”
 - Provides one-sided and two-sided P values
 - Does not provide any meaningful estimates or confidence intervals
 - (The sign test can also be performed by creating the differences, changing the zeroes to missing, and then using “`bitest`”)

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Sign Test: Stata Example

- Example: Change in plasma beta-carotene in placebo group

```
. signtest carot3=carot0 if dose==0
```

```
Sign test
```

sign	observed	expected
positive	1	3.5
negative	6	3.5
zero	0	0
all	7	7

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Sign Test: Stata Example

```
One-sided tests:
```

```
Ho: mdnn of carot3 - carot0 = 0 vs.
```

```
Ha: median of carot3 - carot0 > 0
```

```
Pr(#pos >= 1) = Binomial(n=7, x>=1, p=0.5)= 0.9922
```

```
Ho: median of carot3 - carot0 = 0 vs.
```

```
Ha: median of carot3 - carot0 < 0
```

```
Pr(#neg >= 6) = Binomial(n=7, x>=6, p=0.5)= 0.0625
```

```
Two-sided test:
```

```
Ho: median of carot3 - carot0 = 0 vs.
```

```
Ha: median of carot3 - carot0 ~= 0
```

```
Pr(#pos >= 6 or #neg >= 6) = 0.125038
```

Interpretation

- We can not with 95% confidence reject the null hypothesis that the median change in plasma beta-carotene levels after 9 months of treatment with placebo was 0

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(Wilcoxon) Signed Rank Test

- The sign test is simple to perform, but it ignores a lot of information
 - Intuitively, you would expect that there is some information in the magnitude of the differences as well as the sign
 - For instance, there may be nearly as many negative differences as positive differences, but the positive differences tend to be far larger (in absolute value) than the negative differences

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(Wilcoxon) Signed Rank Test

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- The Wilcoxon signed rank test attempts to use the information about the magnitude of the differences
 - The null hypothesis of the Wilcoxon signed rank test is that
 - the number of positive and negative differences should tend to be equal, and
 - there should be no tendency for the positive differences to be further from (or closer to) zero than the negative differences

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(Wilcoxon) Signed Rank Test

.....

- Basic approach of the signed rank test
 - Compute the differences and rank the absolute value of the differences
 - Sum up the ranks of the positive differences
 - Under the null hypothesis of equality of distributions, the sampling distribution for that sum should be the same as randomly choosing $n/2$ numbers from the integers 1 to n
 - Adjustment for ties and zeroes
 - (Computers can figure this out for us)

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Example of Signed Ranks

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X {9, 7, 4, 2, 37, 9, 7, 4}

Y {3, 8, 4, 5, 7, 5, 9, 5}

Diff {6, -1, 0, -3, 30, 4, -2, -1}

Ranks {7, 2.5, 1, 5, 8, 6, 4, 2.5}

Sum of Positive Ranks : 21

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Summary Measure

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- It is not immediately clear (or easily explained) what aspect of the distributions the signed rank test is comparing
 - Can be significant because
 - Number of positive differences is unusually high
 - Mean positive difference is high
 - It provides some sort of a balance between the two

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Interpretation

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- In any case, it is clear that a significant signed rank test can only be interpreted as a difference in distributions
 - The standard error of the test statistic is based on a permutation distribution, and thus
 - is only testing equality of distributions with the appropriate type I error,
 - but because it is not a consistent test of arbitrary differences between distributions
 - the differences must be something that the signed rank test can detect

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Stata Commands

.....

- Stata has a command to perform the signed rank test
 - “`signrank var1 = var2`”
 - Provides one-sided and two-sided P values
 - Does not provide any meaningful estimates or confidence intervals

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Stata Example

.....

- Example: Change in plasma beta-carotene in placebo group

```
. signrank carot3=carot0 if dose==0
Wilcoxon signed-rank test
   sign |      obs   sum ranks   expected
-----+-----
positive |         1         1         14
negative |         6        27         14
zero     |         0         0          0
-----+-----
      all |         7        28         28
(some purely technical output omitted)
Ho: carot3 = carot0      z = -2.197 Prob > |z| = 0.0280 47
```

Interpretation

.....

- We can with 95% confidence reject the null hypothesis that there was no systematic trend toward increasing or decreasing plasma beta-carotene levels after 9 months of treatment with placebo
 - (Note that we were able to reject the null with the signed rank, but not the sign test.)

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Comparing Proportions: Matched Samples

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Matched Binary Data

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- In some studies, we make comparisons of proportions across samples which are not independent
 - E.g., Cross-over studies
 - Relief of headaches from aspirin vs Tylenol
 - Each subject receives each treatment (in random order)
 - E.g., Ophthalmology studies
 - Cure of conjunctivitis: new treatment vs placebo
 - Each subject receives each treatment (randomize₅₀ which eye receives new treatment)

Presentation of Data

.....

- We tend to alter the format of contingency table to reflect the matched data
 - Instead of response by group, we display concordance of response in each group

	Response					Resp on Plc			
		+	-			+	-		
Treatment	New	<i>r</i>	<i>s</i>	<i>n</i>	Resp on New	+	<i>a</i>	<i>b</i>	<i>r</i>
	Plc	<i>t</i>	<i>u</i>	<i>n</i>		-	<i>c</i>	<i>d</i>	<i>s</i>
		<i>m</i> ₀	<i>m</i> ₁	<i>n</i>		<i>t</i>	<i>u</i>	<i>n</i>	

Estimate

.....

- Usual estimate of difference of proportions

		Resp on Plc		
		+	-	
Resp on New	+	<i>a</i>	<i>b</i>	<i>r</i>
	-	<i>c</i>	<i>d</i>	<i>s</i>
		<i>t</i>	<i>u</i>	<i>n</i>

Estimated difference in proportions

$$\frac{r}{n} - \frac{t}{n} = \frac{b - c}{n}$$

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Analysis of Data

- The analysis of the matched data can proceed along two lines
 - Least frequently used
 - Compare proportion with response in each group taking matching into account
 - Analogous to paired t test (which would be a valid test in large samples)
 - Most often used: McNemar's test
 - Focus on the "discordant pairs" only
 - Evaluate whether discordant pairs are evenly distributed between (+, -) and (-, +)

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McNemar's Test: Rationale

- If response were equal in the two groups, discordant pairs should be equally likely to be in either order
 - Condition on the number of discordant pairs
 - Intuitively, the number of discordant pairs does not contribute much information as to which group does better
 - Under the null hypothesis, the discordant pairs should be equally likely to be in either the "b" or the "c" cell of the contingency table⁵⁴
 - Use the one sample test of a binomial proportion

McNemar's Test

- One sample binomial test

	Resp on Plc			
	+	-		
Resp on New	+	a	b	r
	-	c	d	s
	t	u		n

If response rates are equal for both treatments,
under the null we would have binomial distribution

$$b \sim B(b+c, 0.5)$$

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Stata: Exact McNemar's

- Example: Prevalence of edema vs ascites in liver data
 - Are ascites and edema equally prevalent?
 - Stata does not perform McNemar's using exact distributions, but we can get it to perform the test quite easily

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Stata: Exact McNemar's

```

.....
table edema ascites
-----+-----
          | ascites
edema |    0    7
-----+-----
          |
0 | 268    7
          |
1 |   20   17
-----+-----

```

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Stata: Exact McNemar's

```

.....
. bitesti 27 7 0.5
N   Obs k   Exp k   Assumed p   Observed p
-----+-----
27   7   13.5  0.50000   0.25926

Pr(k>= 7)           = 0.9970   (one-sided test)
Pr(k<= 7)           = 0.0096   (one-sided test)
Pr(k<= 7 or k>= 20) = 0.0192   (two-sided)

```

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McNemar's Test

- Test statistic can be based on asymptotic distribution
 - Standardized Z statistic or (more commonly) a chi squared statistic

Under the null we would have binomial distribution

$$b \sim B(b+c, 0.5)$$

$$Z = \frac{\frac{b}{b+c} - 0.5}{\sqrt{0.25/(b+c)}} \stackrel{H_0}{\sim} N(0,1) \quad \chi^2 = Z^2 = \frac{(b-c)^2}{(b+c)}$$

Stata: Large Sample

- Stata uses asymptotic theory
 - “mcc casevar ctrlvar”
 - mcc = matched case-control
 - Labels are by “Cases” and “Controls”
 - Provides two-sided P-values
 - Provides confidence interval for difference in proportions

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Stata Commands: Example

- Prevalence of edema vs ascites in liver data

```
mcc edema ascites
```

Cases	Controls		Total
	Exposed	Unexposed	
Exposed	17	20	37
Unexposed	7	268	275
Total	24	288	312

McNemar's chi2(1)= 6.26 Pr>chi2= 0.0124

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Stata Commands: Example

- Prevalence of edema vs ascites in liver data

```
Proportion with factor
```

Cases	Proportion	[95% CI]
Cases	.1186	
Controls	.0770	

```
-----
```

	difference	ratio	rel. diff.	odds ratio
	.0417	1.5467	.0451	2.8571
	.0061	1.0954	.0106	1.1605
	.0772	2.1698	.0797	7.9971 (exact)

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Compare Paired t Test

```
ttest edema=ascites
```

Paired t test Number of obs = 312

Variable	Mean	St Err	t	P> t	[95% CI]
edema	.1186	.0183	6.469	0.0000	.0825 .1547
ascites	.0769	.0151	5.091	0.0000	.0472 .1067
diff	.0417	.0165	2.523	0.0121	.0092 .0742

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Compare Paired t Test

Degrees of freedom: 311

Ho: mean diff = 0

Ha: diff < 0	Ha: diff ~= 0	Ha: diff > 0
t = 2.523	t = 2.523	t = 2.523
P < t = 0.9939	P > t = 0.0121	P > t = 0.0061

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Comments

- It is useful to highlight the difference between the questions answered by the chi square test and McNemar's test
 - Consider test of edema and ascites
 - McNemar's test
 - Are ascites and edema equally prevalent?
 - Chi square test
 - Does the prevalence of ascites differ between subjects with and without edema?

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Sign Test vs McNemar's Test

- McNemar's test is just the sign test performed on binary data
 - The sign test is a more general description of the procedure, and thus I prefer using that name even when using binary data
 - Hence, I introduced the word "McNemar" only because you will sometimes see it referred to in the literature
 - I wish the word "McNemar" would disappear from the literature (my brain is full)

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