Multiple Comparisons

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Sponsored by the Clinical and Translational Science Institute (CTSI) and the Department of Population Health / Division of Biostatistics
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Outline

• Introduction/Motivating Example
• Review of Hypothesis Testing
• Multiple Testing Strategies
• Philosophical Issues
• Specific Examples:
  – Multiple Outcomes/Timepoints
  – Multiple Groups
  – Subgroup Analysis in Clinical Trials
  – Large multiplicity problems
  – Interim Analyses
Or, Torturing the Data until it Confesses
Motivating Example

• Panic Disorder study to measure effectiveness of a drug treatment compared to control

• Four outcomes studied
  – Severity of anticipatory anxiety, p=0.04
  – Total number of panic attacks, p=0.10
  – Severity of phobic avoidance, p=0.72
  – Global assessment of patient, p=0.38

• Conclusion based on p<0.05: Drug has a statistically significant effect on severity of anticipatory anxiety
Multipliclity Problem

- Four outcomes were studied
  - Increased chance of finding at least one false significant finding the more tests you perform
  - Is the impact of treatment on severity of anticipatory anxiety "real" or is it an artifact of performing multiple tests?
- Extreme setting: Keep examining more and more outcomes until you find one with a significant p-value
Review of Hypothesis Testing

- Null Hypothesis $H_0$: Typically no difference between groups or no association
- Alternative Hypothesis $H_1$: Research hypothesis, there is a difference or association between groups

- Compute $p$-value: Likelihood of obtaining an observed difference or one more extreme if the null hypothesis were true (i.e. by chance alone)
- Compare $p$-value to significance level $\alpha$: if $p<\alpha$, then reject $H_0$
Types of Errors

Truth about Population

<table>
<thead>
<tr>
<th></th>
<th>$H_0$ is True</th>
<th>$H_0$ is NOT True</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accept $H_0$</td>
<td><img src="%E7%A5%9E%E7%A7%98%E5%9B%BE%E6%A0%87" alt="Smiley" /></td>
<td><img src="%E7%A5%9E%E7%A7%98%E5%9B%BE%E6%A0%87" alt="Sad" /></td>
</tr>
<tr>
<td>Reject $H_0$</td>
<td><img src="%E7%A5%9E%E7%A7%98%E5%9B%BE%E6%A0%87" alt="Sad" /></td>
<td><img src="%E7%A5%9E%E7%A7%98%E5%9B%BE%E6%A0%87" alt="Smiley" /></td>
</tr>
</tbody>
</table>

$\alpha =$ probability of Type I error (level of significance)

$\beta =$ probability of Type II error

$1 - \beta =$ Power
# Testing Multiple Hypotheses

- **K independent tests with type I error 0.05**

<table>
<thead>
<tr>
<th>K</th>
<th>Probability of at least one type I error</th>
<th>Expected number of type I errors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>0.18</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>0.40</td>
<td>0.5</td>
</tr>
<tr>
<td>50</td>
<td>0.92</td>
<td>2.5</td>
</tr>
<tr>
<td>100</td>
<td>0.99</td>
<td>5</td>
</tr>
<tr>
<td>1000</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>10000</td>
<td>1</td>
<td>500</td>
</tr>
</tbody>
</table>
Implications for our Example

• There is a 18% chance of obtaining a statistically significant result among our four endpoints even if there was no effect of treatment on the drug.
• P=0.04 seems less convincing given the context of multiple comparisons
• How do we incorporate the impact of multiple testing on our inference?
Multiple Testing Strategies

• Perform less tests
• Transparency
• Cautious in interpretation
• Ad-hoc adjustment: Use significance level of 1% rather than 5%
• Control a different error rate which incorporates the number of tests performed
  – Familywise error rate (FWE): Probability of at least one type I error among all tests performed
  – False Discovery Rate (FDR): Expected proportion of “False discoveries” out of the total significant findings
## Error Rates

<table>
<thead>
<tr>
<th>Decision</th>
<th>True Null Hypotheses</th>
<th>False Null Hypotheses</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accepted Hypotheses</td>
<td>A (😊)</td>
<td>B (🙁)</td>
<td>M (N)</td>
</tr>
<tr>
<td>Rejected Hypotheses</td>
<td>C (🙁)</td>
<td>D (😊)</td>
<td>N (N)</td>
</tr>
<tr>
<td>Total</td>
<td>T (N)</td>
<td>F (N)</td>
<td></td>
</tr>
</tbody>
</table>

FWE = P(C \geq 1); FDR = Average of (C/N)
Controlling the FWE

- Overall test of whether there is an effect of treatment on all outcomes at once
  - Only examine individual outcomes if overall test is significant (weak control)

- Adjust significance level (equivalently, p-value) for number of tests performed (K)
  - Bonferroni procedure: Use significance level=$\alpha/K$, or multiply all p-values by K
  - Other, more efficient procedures available: See a statistician
  - Strong control
Controlling the FDR

- Benjamini-Hochberg procedure
  - 5% FDR: On average, 5% of your significant findings will be false
  - Order the p-values
  - Compare $i^{th}$ smallest p-value to $i \times 0.05/K$
FWE vs. FDR

• Example comparing 2 groups on a psychological scale which has 14 different subscales
  – Comparison of groups for each subscale
  – 14 tests
<table>
<thead>
<tr>
<th>Ordered p-value</th>
<th>Benjamini-Hochberg threshold</th>
<th>Bonferroni threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00039</td>
<td>0.0036</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.00103</td>
<td>0.0071</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.00159</td>
<td>0.0107</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.00164</td>
<td><strong>0.0143</strong></td>
<td>0.0036</td>
</tr>
<tr>
<td>0.00765</td>
<td>0.0179</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.0196</td>
<td>0.0214</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.0237</td>
<td>0.0250</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.0310</td>
<td>0.0286</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.101</td>
<td>0.0321</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.157</td>
<td>0.0357</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.284</td>
<td>0.0393</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.542</td>
<td>0.0429</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.543</td>
<td>0.0464</td>
<td>0.0036</td>
</tr>
<tr>
<td>0.793</td>
<td>0.0500</td>
<td>0.0036</td>
</tr>
</tbody>
</table>
FWE vs. FDR

• FDR controlling procedures are generally more powerful than FWE controlling procedures
  – More likely to detect real differences as significant
  – Allows for an acceptable rate of type I errors among significant findings
  – Not appropriate when strict control of any type I errors is desired
  – Exploratory setting
  – Large scale multiple testing problems: Genetics, Imaging

• FWE controlling procedures:
  – Strict control of risk of any type I errors
  – More appropriate in confirmatory or regulatory setting
What about confidence intervals?

"When you say you're 95% confident... just what are you inferring?"
Confidence Intervals

- Equivalence between hypothesis testing and confidence intervals
- Null Hypothesis: mean difference = 0
  - Construct a confidence interval for the mean difference and see if it contains the null value of 0
  - Correspondence between type I error rate of hypothesis test (5%) and confidence level of interval (95%)
Multiple Confidence Intervals

- 95% Confidence Interval: Probability the interval contains the true parameter is 95%
- 95% *Simultaneous* Confidence Interval: Probability that all intervals contain the true parameters is 95%
- Wider than individual CI’s
- Direct correspondence to FWE
- If you adjust your p-values, you should also adjust your Confidence Intervals
Philosophical issues

• Differing opinions on whether or when adjustment is needed
• Choice of family of hypotheses to adjust for is arbitrary, and results are very sensitive to this choice
  – Choose small, more focused families, specified a priori (in writing) to avoid cheating
• Increase in type II errors due to adjustment (loss of power or ability to detect real differences)
  – Use an appropriate and powerful testing procedure (see statistician)
• Argue for unadjusted analysis, but with full disclosure of data analysis procedures
  – Difficult for reviewers to evaluate
Philosophical issues

• Need for adjustment, and best method of adjustment, is often scenario dependent
• Things to consider (Westfall et al., 1999)
  – Is it plausible that many of the null hypotheses might be true?
  – Do you want to ensure reproducibility, or be able to claim that an identified significant finding is in fact real?
  – Do you want to heavily mine the data to find a “significant” result?
  – Is your study expensive and unlikely to be repeated before serious actions are taken?
  – Is there an important cost associated with type I errors?
Multiple Outcome Variables

- Limit number of outcomes
- If control of type I error is desired
  - Outcomes are often correlated
  - Bonferroni method is conservative
  - Overall test of no difference on any outcomes is more powerful (Multivariate methods)
### Multiple Outcome Variables

- Panic Disorder Motivating Example: Bonferroni adjustment

<table>
<thead>
<tr>
<th>Outcome</th>
<th>P-value</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity of anticipatory anxiety</td>
<td>0.04</td>
<td>0.16</td>
</tr>
<tr>
<td>Total number of panic attacks</td>
<td>0.10</td>
<td>0.40</td>
</tr>
<tr>
<td>Severity of phobic avoidance</td>
<td>0.72</td>
<td>1.00</td>
</tr>
<tr>
<td>Global assessment of patient</td>
<td>0.38</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Multiple Outcome Variables

• Overall test:
  – Null hypothesis: No difference between treatment and control on any of the 4 endpoints
  – p=0.20
• No evidence of a difference on any of the endpoints
• Ignore significant p-value (p=0.04) on severity of anticipatory anxiety
  – Likely attributable to multiple tests
Multiple Outcome variables

• Confirmatory clinical trials
  – Strict control of type I error rate desired
  – Primary endpoint:
    • usually one
    • pre-specified in protocol
  – Secondary endpoints:
    • Exploratory
    • Explanatory of findings in primary endpoint
  – No adjustment needed for primary endpoints
Multiple Outcome variables

• Composite Endpoints:
  – Combine multiple endpoints into one to reduce multiple comparisons problem
  – Cardiovascular: Myocardial infarction, stroke, or cardiovascular death
  – Cancer: Progression-free survival
  – Potential difficulties in interpretation
Multiple Time Points

- Repeated Measures study of dental measurements in boys and girls (Pothoff and Roy, 1964) at ages 8, 10, 12, 14
Multiple Time Points

- Separate t-tests at each time point comparing boys and girls are susceptible to multiplicity issues
- Fit a linear growth curve
  - Reduces number of comparisons from 4 time points to 2 parameters (slope and intercept)
- Overall test
  - Null Hypothesis: No difference between boys and girls at any age
  - Comparisons at each age only performed if overall test is significant
**Multiple Time Points**

- Comparisons between Boys and Girls at each age

<table>
<thead>
<tr>
<th>Age</th>
<th>Mean difference (B-G)</th>
<th>p-value</th>
<th>Bonferroni Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1.69</td>
<td>0.066</td>
<td>0.264</td>
</tr>
<tr>
<td>10</td>
<td>1.58</td>
<td>0.084</td>
<td>0.336</td>
</tr>
<tr>
<td>12</td>
<td>2.63</td>
<td>0.005</td>
<td>0.02</td>
</tr>
<tr>
<td>14</td>
<td>3.38</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Multiple Time Points

- Overall test:
  - Null Hypothesis: No differences between boys and girls at any age
  - p=0.006
  - Since this is significant, we look at unadjusted p-values for each time point
  - Significant differences between boys and girls at Age 12 (p=0.005) and Age 14 (p<0.001)
- Both multiplicity adjustments give similar findings
- Final Conclusion: Mean dental measurements are significantly different between boys and girls at ages 12 and 14
Multiple Groups

• Comparison of B-leucocyte counts in 51 subjects with colorectal cancer (Werther et al., 2002)

• Four classifications of cancer patients:
  – Duke’s Classification A, B, or C
  – Group D=patients with disease which had not been completely resected
  – 6 pairwise comparisons
Multiple Groups

Boxplot of B_Leucocyte count

- Group A
- Group B
- Group C
- Group D
Multiple Groups

- Unadjusted p-values for pairwise comparisons (row vs. column)

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.045</td>
<td>0.098</td>
<td>0.062</td>
</tr>
<tr>
<td>B</td>
<td>0.683</td>
<td>0.891</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>0.638</td>
<td></td>
</tr>
</tbody>
</table>

- Significant difference between Classification A and B (p=0.045)
Multiple Groups

- Overall ANOVA F-test has p=0.191
- Ignore significant result in pairwise comparisons when the overall test is not significant
- Final Conclusion:
  - No significant difference in B-leucocyte counts between cancer groups
- For strict control of type I error rate, standard method is Tukey test
  - More powerful than Bonferroni
Multiple groups

- Adjusted p-values for pairwise comparisons using Tukey test

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.183</td>
<td>0.342</td>
<td>0.237</td>
</tr>
<tr>
<td>B</td>
<td>0.976</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>0.964</td>
<td></td>
</tr>
</tbody>
</table>

- No significant differences among cancer groups, since all adjusted p-values are >0.05.
Subgroup Analysis

Is effect of treatment in a clinical trial homogeneous across all patients in that trial?

Example 1: ISIS-2 Trial: 17000 patients with AMI randomized to placebo vs. aspirin (also streptokinase) (Lancet, 1988)
- Mortality within 1 month: 9% (aspirin) vs. 12% (placebo), p<0.001
- Investigators were urged (by editors) to conduct nearly 40 subgroup analyses
- Investigators agreed on condition that they could conduct their own subgroup analysis to illustrate unreliability of subgroup findings
Subgroup Analyses

- Subgroup defined by astrological sign

<table>
<thead>
<tr>
<th>Astrological sign</th>
<th>Aspirin</th>
<th>Placebo</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Libra or Gemini</td>
<td>150</td>
<td>147</td>
<td>NS</td>
</tr>
<tr>
<td>Others</td>
<td>654</td>
<td>869</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

- Increased variability of results just due to chance when you look at a lot of subgroups.
- Excess of type II errors due to multiple comparisons.
Subgroup Analysis

- Example 2: Effect of new vs. standard antibiotic on febrile morbidity in four age strata and overall

<table>
<thead>
<tr>
<th>Age</th>
<th>Rate ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-24</td>
<td>1.4</td>
<td>(0.6-3.2)</td>
</tr>
<tr>
<td>25-29</td>
<td>1.2</td>
<td>(0.4-3.1)</td>
</tr>
<tr>
<td>30-34</td>
<td>0.3</td>
<td>(0.1-0.9)</td>
</tr>
<tr>
<td>35-39</td>
<td>1.1</td>
<td>(0.5-2.5)</td>
</tr>
<tr>
<td>Overall</td>
<td>0.9</td>
<td>(0.6-1.4)</td>
</tr>
</tbody>
</table>

- Analysis in 4 subgroups inflates the type I error rate
Subgroup Analysis

• Proper assessment of subgroups
  – Perform overall test of whether the subgroups differ
  – Null Hypothesis: treatment effect is the same for each subgroup (No interaction between subgroup and treatment)
  – Only look at each subgroup if interaction test is significant

• Example 2: Interaction test: $p=0.103$, no evidence of interaction
  – Subgroup finding in age 30-34 group is likely due to chance
Subgroup Analysis

• Subgroup analyses are discouraged and prone to overinterpretation

• Recommendations
  – Perform interaction test: only look at each subgroup separately if interaction test is significant
  – Confine to primary outcome and limited number of subgroups
  – Prespecified in protocol
  – Consider biological plausibility
  – Report all subgroup analyses done – may need to adjust for multiple subgroup variables (age, sex, disease status)
  – Generally considered EXPLORATORY
Large Multiplicity Problems

• Genetics and microarrays
  – Thousands of genes assessed for their association with phenotype
  – Hypothesis test performed for each gene
  – If each test is performed at the 5% significance level, we would expect 50 false findings in 1000 tests.
Large Multiplicity Problems

- Functional Magnetic Resonance Imaging
  - Experiment performed and Blood Oxygen Level Dependent (BOLD) response assessed at each of thousands of voxels or points in the brain
  - Example: Finger-tapping experiment
  - Null hypothesis: Mean BOLD signal while tapping is the same as the Mean BOLD signal while not tapping
  - T-test performed at each point in the brain
T statistic image
Threshholded at 1.96
5% FWE Threshold (Bonferroni)
5% FDR Threshold
Large Multiplicity Problems

• Unadjusted method: Too many false positives
• FWE adjustment: Loss of power to detect real effects
• FDR procedures: Compromise
  – Low rate of false positives relative to true discoveries
  – Improved power relative to FWE adjustment
Interim Analyses

• Clinical Trials are routinely monitored for safety by a DSMB
  – Meets every 6-12 months to review data
• Justifications for early termination of study
  – Unacceptable toxicity
  – Accrual problems
  – Efficacy
  – Futility
Interim Analyses

• Repeated tests for efficacy can inflate the type I error rate

<table>
<thead>
<tr>
<th># of looks</th>
<th>Type I error rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>0.11</td>
</tr>
<tr>
<td>10</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Interim Analyses

• Plan must be specified in the protocol
• Adjust significance level at each interim analysis
• For example, with 3 looks at the data

<table>
<thead>
<tr>
<th>Interim analysis</th>
<th>Adjusted significance level (O’Brien-Fleming)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0005</td>
</tr>
<tr>
<td>2</td>
<td>0.014</td>
</tr>
<tr>
<td>3 (final)</td>
<td>0.045</td>
</tr>
</tbody>
</table>
Summary

• The more tests you do the more likely you are to find a significant result
• Restrict or prioritize the number of tests
• Be cautious and aware when interpreting results
• Explicit corrections for multiple testing are available
  – Strengthen evidence for significant research findings
  – Loss of power
• Need an appropriate
  – Family of hypotheses
  – Type of error rate
Resources

• The Clinical and Translation Science Institute (CTSI) supports education, collaboration, and research in clinical and translational science: www.ctsi.mcw.edu

• The Biostatistics Consulting Service provides comprehensive statistical support http://www.mcw.edu/biostatsconsult.htm
Free drop-in consulting

- MCW/Froedtert/CHW:
  - Monday, Wednesday, Friday 1 – 3 PM @ CTSI Administrative offices (LL772A)
  - Tuesday, Thursday 1 – 3 PM @ Health Research Center, H2400
- VA: 1st and 3rd Monday, 8:30-11:30 am
  - VA Medical Center, Building 70, Room D-21
- Marquette: 2nd and 4th Monday, 8:30-11:30 am
  - Olin Engineering Building, Room 338D
### Upcoming Lectures

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 14</td>
<td>7AM</td>
<td>Aniko Szabo, PhD</td>
<td>Concepts on the Way from Data to Decisions</td>
<td>NT22009</td>
</tr>
<tr>
<td>September 24</td>
<td>1:30PM</td>
<td>Brent Logan, PhD</td>
<td>Designing Clinical Trials</td>
<td>CHW Auditorium</td>
</tr>
<tr>
<td>August 26</td>
<td>8:50 AM</td>
<td>Prakash Laud, PhD</td>
<td>Concepts on the Way from Data to Decisions</td>
<td>TBA</td>
</tr>
<tr>
<td>September 30</td>
<td>8:50 AM</td>
<td>Jennifer Le-Rademacher, PhD</td>
<td>Statistics, Probability and Diagnostic Medicine</td>
<td>TBA</td>
</tr>
</tbody>
</table>

For locations that are TBA please check the website below two weeks prior to the lecture date:

[http://www.mcw.edu/biostatistics/CalendarCurrentEvents/SeminarSeries.htm](http://www.mcw.edu/biostatistics/CalendarCurrentEvents/SeminarSeries.htm)