Contents

Multiplicity &c.

Multiplicity & Interim Analysis

- **Books**
  - **Andersen, B. (1990)** *Methodological Errors in Medical Research.* Blackwell
- **Papers**
  - ICH E9 Expert Working Group. (1999) Statistics in Medicine, 18, 1905-42.
  - Philips, Alan & Haudiquet, Vincent (2003) Statistics in Medicine, **22**, 1–11

- Multiplicity arises in
  - Multiple end-points
  - Subgroup analyses
  - Interim testing
  - Repeated Measures
  - &c.
- Problem of repeated significance tests
  - May inflate risk of false positive
    - i.e. overall significance level

- **Example:** Effect of new dietary control regime.
- **Data:** 250 subjects:
  - Weight loss at end of week.
    - Data in kg.
- Paired t-test gives p–value of 0.067
- **Not quite significant at the 5% level !**

- Can anything be done to ‘squeeze’ a significant result out of this expensive study ?????
  - we’ve been told we cannot change our mind and use a one-sided test instead!
- subgroup data by Sign of the Zodiac:–

- p-value for Aries is 0.019
  - With mean weight **loss** of 0.5kg
- p-value for Taurus is 0.099
  - With mean weight **gain** of 0.3kg
- **Conclusions:**
  - Diet successful for those under Aries
  - Taurus subjects are perverse

- Clearly a ***False Positive Result***
- Fallacy arises because of **selecting most significant result.**
  - (data are artificial, but not very)
    - useful device to try if pressed to perform post-hoc subgroup analysis (c.f. Richard Peto)

- Statistical tests make mistakes
  - Declare a real difference exists
    - but in fact the observed difference is due to natural chance variation
  - Risk controlled ***for each individual single test***
    - *significance level* of the test or the *p-value*
  - if **many** separate significance tests then difficult to control overall risk of declaring *at least one false positive somewhere*

- 5% test then 95% chance of no mistake
  - Two 5% tests then 95%×95% (= 90.25%) of no mistake on either
  - So 10% risk of one or other (or both) giving a false positive
  - i.e. overall significance level is ~10%

- ***c.f. Normal Ranges*** in clinicochemcal tests
  - A 'normal person' is one who has not been sufficiently investigated.
- A *normal range* comprise 95% of values
  - 100 normal persons evaluated then only 95 of them will 'normal'
  - If then subjected to another **independent** test only 90 will remain as 'normal'

- **Multiplicity**:
  - 10 independent tests at [nominal] 5%
  - H_0 true in all (i.e. no difference)
  - Chance of rejecting *at least* 1 is 40%
  - **SO** reduce nominal level in each to control overall significance level

- **Bonferroni correction**
  - k tests, want overall level to be α
- Take nominal level on each test as α/k
- **Example:**
  - 5 separate tests
  - Overall 5% level of significance wanted
  - Declare a result if any test nominally significant at the 5%/5=1% significance level

- **Example:**
  - 25 tests are to be performed
  - overall level of 1% intended
  - so each should be run at a nominal level of 1/25=0.04%
  - i.e. a result should not be claimed unless p < 0.0004 in any one of them

- **Example:**
  - 12 tests have been performed
  - smallest p-value is 0.019
  - What is the overall level of significance?
  - Bonferroni method says overall level is 12×0.019 = **0.228**
    - This is the Signs of the Zodiac example
    - i.e. no worthwhile evidence of any birth sign being particularly suited to dieting
      - (see again later)

- Bonferroni method typically **very conservative**
  - i.e. less likely to be able to declare a real difference exists even if there is one
  - But is 'safe'
    - i.e. you preserve your scientific reputation by avoiding making mistakes but at expense of failing to discover something scientifically interesting

- **Multiple End-points**
  - e.g. pulse rate, systolic & diastolic blood pressure sitting,standing & supine before & after exercise
  - Separate tests high risk of false positives

- **Remedies:**
  - Bonferroni correction
  - choose primary outcome measure
  - multivariate analysis
- **NB:** Bonferroni **very** conservative
  - multiple outcome measures likely to be ***highly correlated***
  - standing systolic BP will give similar evidence to sitting BP

- Very frustrating if you had considered 20 highly correlated measures
  - each gives nominal p-value of 0.01
  - Bonferroni says can only claim an overall p-value of 0.2
  - Would have been better not to have measured the other 19

- Better is to define ***primary outcome***
  - perhaps 2 or 3 secondary measures
  - ***Must be stated in the protocol***
    - medical expertise
    - initial results from a pilot study
  - Other measures (e.g. lab results) should be scrutinised
    - report causes for concern

- **Multivariate Analysis**
  - Makes proper allowance in the analysis for correlated observations
  - There are multivariate equivalents of standard univariate statistical analyses
    - Student's t–test ←→ Hotelling's T^2–test
    - ANOVA ←→ MANOVA
      - **M**ultivariate **An**alysis **o**f **Va**riance
      - Wilks' test or Lawley-Hotelling test

- **Advantage** of multivariate analysis
  - handle all measures simultaneously
  - return a single p–value
- **Disadvantage**
  - difficulty of interpreting the nature of the difference detected
- Many MV procedures in stats packages
  - Advice must be to use them with caution unless experienced help is to hand

- **Cautionary Examples**
  - ref: Br J Clin Pharmacol [Suppl.], 1983, **16**: 103
  - effect of midazolan on sleep
  - table of 2×9 tests of significance on measures of platform balance made at various times
    - ***repeated measures analysis*** (see later)

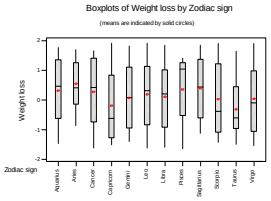
- **Cautionary Examples**
  - ref: Basic Clin Med 1981, **15**: 445
  - double-blind controlled clinical trial to treat rheumatoid arthritis
  - several end–points repeated at various timepoints and various subdivisions
  - 850 pairwise comparisons were made
    - t–tests and Fisher's exact test
  - 48 of these gave p-values < 0.05
  - But expect 5% of 850 = 850/20 = 42.5 so finding 48 is not very impressive

- Andersen quotes
  - *The Lancet* (1984, **ii**: 1457)
  - Moreover, submitting a larger number of factors to statistical examination not only improves your chances of a positive result but also enhances your reputation for diligence

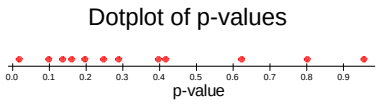
- **Subgroup analyses**
  - Similar problems with subgroups
  - ♦ Need to specify which subgroups of particular interest in protocol
  - ♦ If none in particular then
    - Bonferroni adjustment
    - Analysis of Variance
    - Follow-up tests for multiple comparisons

- One-way ANOVA generalisation to several samples of a two−sample t−test
  - ♦ tests differences between subgroups
    - tests null hypothesis that all subgroups have the same mean *vs* one or more is different
  - ♦ If effect exhibited in only one of several subgroups then one (or more) of the subgroups is different from the rest so test this with ANOVA
    - Follow-up tests to identify which is of interest
    - Tukey's / Dunnett's / Neuman-Keuls /……

- **Example:** Signs of Zodiac (see notes)
  - ♦ p-value for differences in weight loss between Zodiac signs is 0.405
  - ♦ No evidence of difference so follow-up tests not really appropriate
    - (but see example in notes)



- Can also look at 12 separate p-values



  - ♦ If any evidence that some groups were shewing an effect then some of them would be clustered towards near 0.0 and not evenly spread out

- Example (Lee et al, *Circulation*, 1980)
  - ♦ 1073 subjects randomized in two groups
    - No overall significance
    - 6 [post-hoc] subgroups defined
    - One of these produced significance at nominal 2.5% level (p=0.023)
    - Medical reason for expecting this subgroup to be different

- But:—
  - ♦ In fact, no difference between 'treatments'
  - ♦ All patients were treated in the **SAME** way
  - ♦ Groups were just random allocations
- i.e. a ***false positive effect***

- **Cautionary Example** (see notes)
  - ref: N Engl J Med 1978, **298:** 647
  - Complex study on age at presentation of European, black and Latino men & women in an anaemia study
  - Needs a 3-way ANOVA to investigate interactions between gender and race and age.

- **Interim analyses**
  - Desirable in long trial
    - Check protocol compliance
    - Side effects?
    - Feedback
      - (maintains interest)
    - Detect big effects quickly

- However, multiplicity problems:
  - Specify in protocol
  - Adjust nominal significance levels
- Bonferroni too conservative
  - (accumulating data)

Repeated significance tests on accumulating data

Number of repeated tests at the 5% level	overall significance level
1	0.05
5	0.14
10	0.19
100	0.37

Nominal significance levels required to achieve overall level		
N	α=0.05	α=0.01
2	0.029	0.0056
5	0.016	0.0028
10	0.0106	0.0018

- Comparison of drug combinations CP and CVP in non-Hodgkins lymphoma.
  - Measure: tumour shrinkage
  - Trial: over 2 years, about 120 patients.
  - Five interim analyses planned, roughly after every 25th result.
    - Table gives numbers of 'successes' and ***nominal*** p-values using a χ^2 test at each stage.

	response rates		
Analysis	CP	CVP	statistic & p-value
1	3/14	5/11	1.63 (p>0.20)
2	11/27	13/24	0.92 (p>0.30)
3	18/40	17/36	0.04 (p>0.80)
4	18/54	24/48	3.25 (0.05<p<0.1)
5	23/67	31/59	4.25 (0.025<p<0.05)

- **Conclusion**:
  - Not significant at end of trial (overall p>0.05) since p>0.016
    - the required nominal value for 5 repeat tests
  - If **NO** interim analyses had been done then conclusion would have been **different**
    - CVP declared significantly better at 5% level

- **Cautionary Example:**
  - ref: Br J Surg, (1974), **61**: 177
  - No significant difference with 49 patients
  - The trial was therefore continued
  - After 100 patients gave result $\chi^2 = 4.675$, d.f. = 1, $p < 0.05$
    - (and the trial was published)
  - Actual nominal p−value is 0.031 > 0.029 so cannot claim overall 5% significance

- Continuing to collect data until a significant result is obtained is clearly dishonest — eventually an apparently significant result will be obtained

- **Repeated Measures**
  - same feature on a patient measured at several time points
    - blood concentration at baseline and at 1, 3, 6, 12 and 24 hours after drug
  - Must not do t-tests at each time point
    - diagrams with mean values of the two treatment groups plotted against time with ***error bars for each mean*** invite the eye to do exactly that

- **Remedies**
  - Bonferroni adjustments
    - Very conservative (high correlation)
  - Multivariate analysis
    - Special techniques for this
  - Construction of summary measures
    - Area under curve
    - Change from base line
    - Mean change
    - ……

- **Miscellany**
  - Post-hoc re-grouping
    - Dangerous to combine small subgroups together after the data have been collected
- Example:
  - Death or survival in 90 days after heart attack
    - 65-69 age group combined with older or younger groups

	placebo	metoprolol	
deaths	62/697 (8.9%)	40/698 (5.7%)	p<0.02
age 40–64	26/453 (5.7%)	21/464 (4.5%)	p>0.2
age 65–74	36/244 (14.8%)	19/234 (8.1%)	p=0.03
	Metoprolol better for elderly?		
age 40–69	51/627 (8.1%)	32/629 (5.1%)	p=0.04
age 70–74	11/70 (15.7%)	8/69 (11.6%)	p>0.2
	Metoprolol better for younger?		

- **Multiple Regression**
  - large regression analyses many explanatory variables
    - ordinary regression
    - logistic regression for success/failure data
    - Cox regression for survival data
  - Need to ensure that effects are not selected just because they are the most significant coefficients

- **Example:**
  - men who did not shave regularly were '70% more likely to suffer a stroke and 30% more likely to suffer heart disease'
    - according to study at the University of Bristol
  - Perhaps from a logistic regression analysis
  - Is diligence in shaving a medically plausible feature to be investigated???
  - How many other variables were included in the study???

- **Summary and Conclusions**
  - Multiplicity can arise in
    - testing several different responses
    - subgroup analyses
    - interim analyses
    - repeated measures
    - &c.
  - The effect of multiplicity is to increase the **overall risk of a false positive (i.e. the overall significance level)**

- Problems of multiplicity can be overcome by
  - Bonferroni corrections
    - Bonferroni typically **very** conservative
  - other adjustments in special cases
    - e.g. for accumulating data in interim analyses where adjusting for multiplicity can have counter-intuitive effects
  - more sophisticated analyses e.g. ANOVA or multivariate methods
- **"If you torture the data often enough it will eventually confess"**