Statistical Considerations

The Use of Mucosal Assays in Microbicide Trials

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Outline

- Design issues:
  - Hypotheses
  - Sampling
  - Sample size/Power
- Analysis issues:
  - Statistical Analysis Plan
  - Multiple Comparisons
  - Dimension Reduction

Design Issues - Hypotheses

- Mucosal assay results in microbicide trials
  - Generally secondary or exploratory endpoints
    - Still deserve well defined hypotheses
  - Numerous hypotheses (this is ok)
- *A priori*: Why do we care about these assay results and what are the hypotheses regarding them?

Design Issues – Sampling Timing

- Timing of sampling and your hypotheses
  - Baseline sampling
    - hypotheses re: within participant changes
  - Longitudinal sampling
    - Sampling frequency, timing addresses hypotheses
      - Acute versus chronic exposure to microbicide

Design Issues – Sample Size/Power 101

- Mucosal assay results in microbicide trials usually limited by available sample size
- Generally 5 relevant variables:
  - Sample size
  - False positive rate (α) – 0.05
  - Power (1-false negative rate) – 80% or 90%
  - Magnitude of effect size (hypothesized)
  - VARIABILITY!

Design Issues – Variability

- Variability
  - Within assay (noise)
  - Within participant
  - Between participant

Design Issues – Variability

- Within assay variability (noise)
  - Consider 3 replicates of one sample

Assay	Replicate 1	Replicate 2	Replicate 3	Standard Deviation
A	10	100	90	49
B	40	60	50	10

  - Assay A will require much larger sample size than assay B to discern a similar magnitude of difference

Analysis Issues – Statistical Analysis Plan

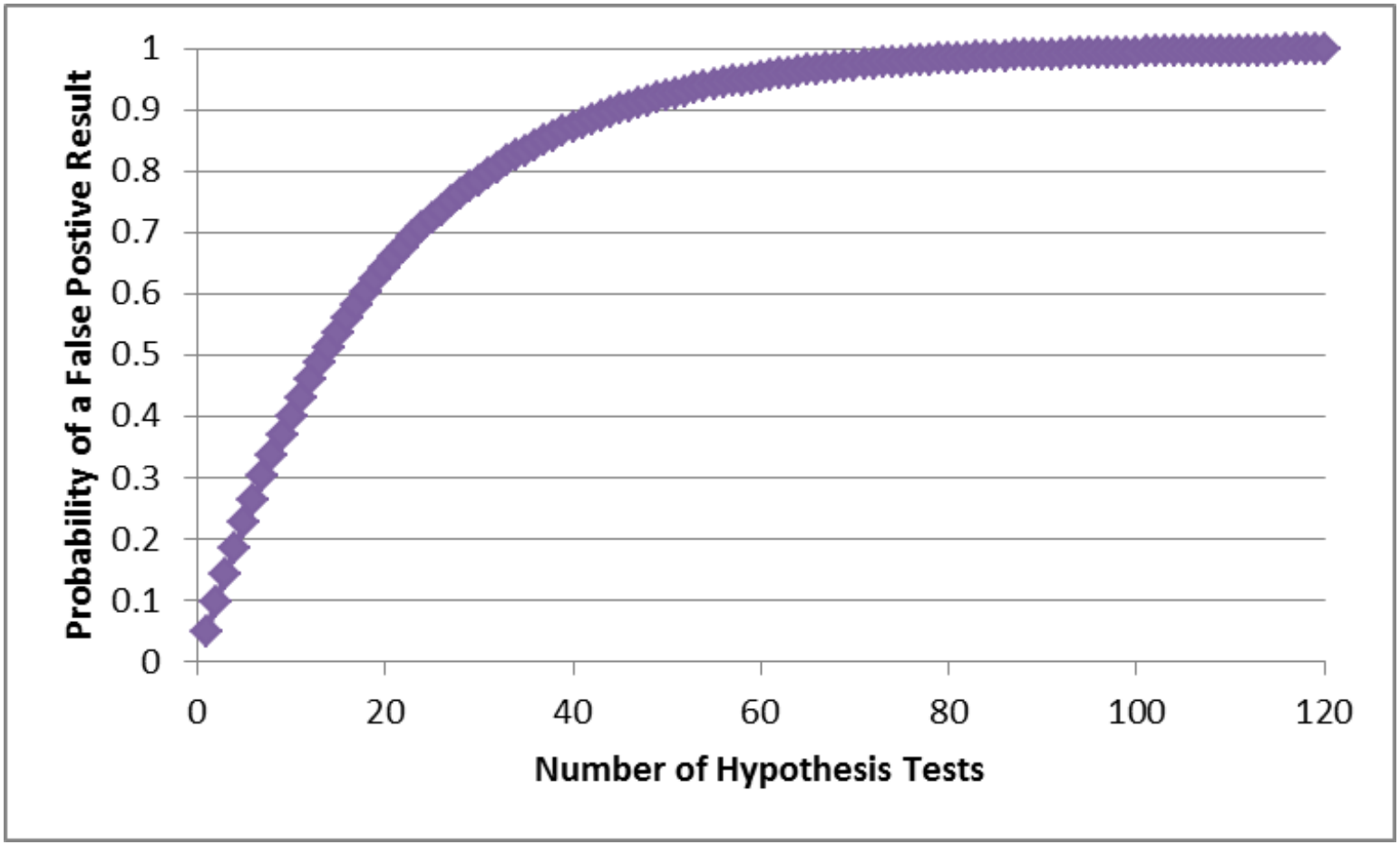
- Statistical analysis plan includes at minimum
  - Hypotheses
  - Endpoints
  - Analysis population description
  - Statistical methods
    - Transformation of variables – Normality or categorization (lower limit of detection)
    - Statistical tests to be used
    - Potential covariates
    - Methods for accounting for multiple comparisons

Analysis Issues – Multiple Comparisons 101

DECISION	TRUTH	
	H_0 True	H_0 False
Do Not Reject H_0	CORRECT 1-α	INCORRECT (false negative) β
Reject H_0	INCORRECT (false positive) α	CORRECT (power) 1-β

Analysis Issues – Multiple Comparisons 101

- Want to control probability of a false positive result (α)



Analysis Issues – Multiple Testing Methods

I can't live with ANY false positive results!

- Methods that control the "Family Wise Error Rate" (FWER) = Pr(at least one false positive)
  - Single step
    - Bonferroni: reject any hypothesis with p-value $\leq \alpha/m$ (m is number of tests)
    - Too conservative – high probability of false negative results
  - Sequential
    - Holm's Method, Simes' Method, others
    - Different criteria for magnitude of p-value rejected
    - Choice depends on correlation of hypothesis tests as well as other factors

Analysis Issues – Multiple Testing Methods

I can live with some false positive results…..

- Methods that control the “False Discovery Rate” (FDR) = proportion of false positives among the set of rejected hypotheses
  - Strive to keep the FDR below a threshold “q” – defined as the q-value
  - Benjamini and Hochberg FDR
  - Storey’s positive FDR (pFDR)

Analysis Issues – Multiple Testing Methods

False Discovery Rate (FDR) versus False Positive Rate (FPR)

DECISION	TRUTH		Total
	H_0 True	H_0 False	
Call H_0 True (do not reject)	95	5	100
Call H_0 False (reject)	**5**	20	**25**
TOTAL	100	25	125

FDR=20% (**5**/**25**)

FPR=5% (5/100)

Analysis Issues – Dimension Reduction

- Numerous mucosal assay outcome variables
  - Are there some variables that cluster together to mark a similar underlying biological mechanism?
- Methods for reducing dimension (combining variables)
  - Principal components analysis
  - Linear discriminant analysis
  - Canonical correlation analysis
  - Others

Analysis Issues – Dimension Reduction

- Example: MTN 004 MTN BSWG Analyses (Pellett Madan, *et al*, 2015)
  - 61 women with 4 visits (baseline, 7 days, 14 days and 21 days)
  - IL-1β, IL-6, IL-12p40, MIP-1α, GM-CSF, lactoferrin and SLPI from cervical swabs
  - **Soluble immune mediator score created using factor analysis with principal components extraction**

Analysis Issues – Dimension Reduction

- Example: MTN 004 MTN BSWG Analyses (Pellett Madan, *et al*, 2015)
  - **Soluble immune mediator score created using factor analysis with principal components extraction**
  - Score used in analyses to see if it was predictive of subsequent endogenous activity against *E. coli*
  - **Dimension reduced from 7 hypothesis tests (7 separate assay results) to 1 (score)** – probability of at least one false positive reduced from ~30% to 5%

Conclusions

- Design:
  - If possible build mucosal assays into study design up front
    - Timing of sampling
    - Sample size/Power
  - DRIVEN BY HYPOTHESES! *A priori*: Why do we care about these assay results and what are the hypotheses regarding them?

Conclusions

- Analysis:
  - Statistical Analysis Plan
    - Multiple testing procedures
    - Possibility of dimension reduction?
  - DRIVEN BY HYPOTHESES! *A priori*: Why do we care about these assay results and what are the hypotheses regarding them?

Acknowledgments

- Fred Hutchinson Cancer Research Center
  - Elizabeth Brown
  - Raphael Gottardo

Design Issues – Sampling Noise

- “Noisy” assays
  - Separate signal from noise
    - Baseline sampling
    - Placebo sampling

Design Issues – Variability

- Within participant variability
  - Consider data on two participants from 3 timepoints for a particular assay

Participant	Time 1	Time 2	Time 3	Standard Deviation
X	10	100	90	49
Y	40	60	50	10

  - Participant X's assay results are much more variable over time than participant Y's. Harder to see a smaller signal in participants like X.