Fundamentals of biostatistics

Sampling and sampling distributions

Lesson 9 – Part 1

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Lesson objectives

- Distinguish between a population parameter and a sample statistic.
- Determine and define an appropriate parameter of interest, based on a research question.
- Demonstrate an understanding of standard error as the standard deviation of the statistic.
- Interpret a confidence interval in context.
- Demonstrate an understanding of the concept of statistical significance.

Parameter and statistic

The relationship between sample **statistics** and population **parameters** is the basis of **statistical inference.**

Parameter	Statistic
Is a fixed numerical value which describes a particular characteristic of a population.	Sample statistics – sample characteristics
Usually not possible to make measurements on every individual in a population.	Sample statistics vary in value from sample to sample.
Characteristics are computed from a random sample.	Sample statistic may be considered to be a random variable.

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Parameter and statistic - examples

- Single population mean
- Single population proportion
- Difference in means
- Difference in proportions
- Odds ratio
- Regression coefficient

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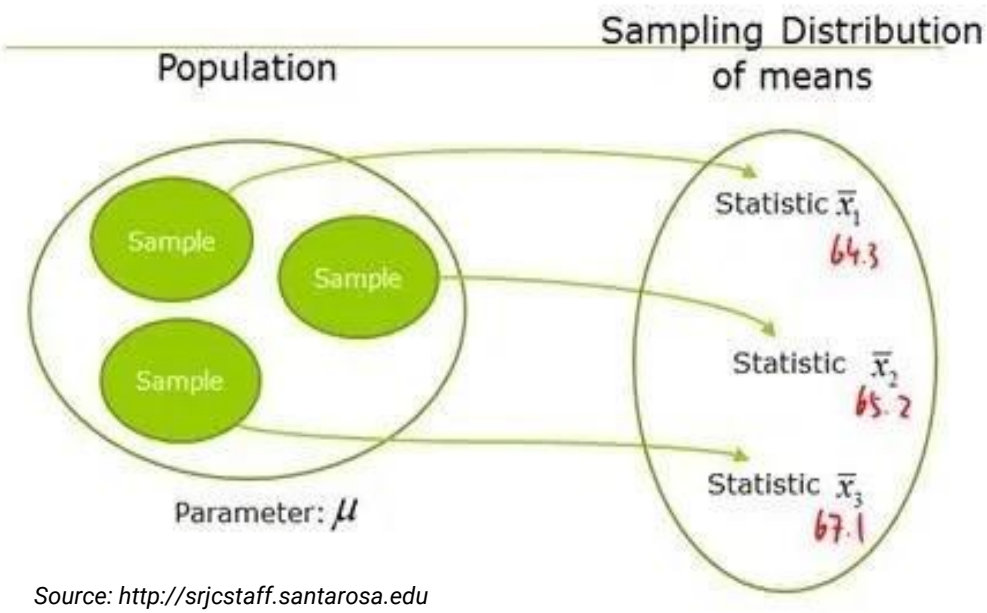
Sampling distribution (1)

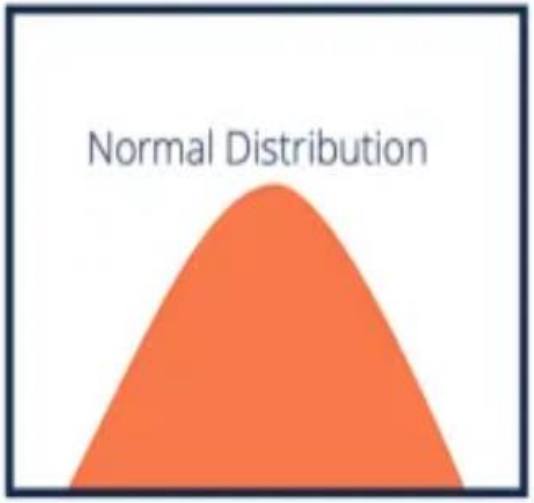
- Statistics follow distributions too.
- But the distribution of a statistic is a theoretical construct.
- Statisticians ask a thought experiment: how much would the value of the statistic fluctuate if one could repeat a particular study over and over again with different samples of the same size?
  - By answering this question, statisticians are able to pinpoint exactly how much uncertainty is associated with a given statistic.

Sampling distribution (2)



Source: http://srjcstaff.santarosa.edu

Distribution of sample means



Source: corporatefinanceinstitute.com

- The characteristics of the sampling distribution serve as an important foundation for **statistical inference.**

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Statistical Inference

The procedure by which we reach **a conclusion about a population** on the basis of the information contained in a sample drawn from that population.

In this section, we will use the knowledge of the properties of the distribution of sample statistic to the process of statistical inference.

The goal is to describe or estimate some characteristics of a random variable e.g. mean using information contained in a sample of observations.

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Confidence intervals (CI)

- Consider the problem of estimating the mean head circumference of a population.
- From a single sample we can calculate the sample mean ($\bar{x}$) which is an estimate of the mean head circumference (μ) of the population.
- However the value of the sample mean will vary from sample to sample.
- We cannot afford to take lots of samples to get a better estimate of the population mean via the mean of the sample means.
- **A confidence interval** takes into account the sample-to-sample variation of the statistic by defining an interval within which the true population parameter is likely to fall.
- CI for statistical significance (if the 95% CI does not cross the null value, it is significant at .05).

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Confidence intervals - example

- A study is conducted to evaluate the effectiveness of amlodipine to reduce systolic blood pressure (SBP) in hypertensive patients. Patients were randomized to receive placebo (n=120) or amlodipine (n=123).
- Summary data

	Mean	SD
Placebo	146.6	13.8
Amlodipine	137.3	10.3
Amlodipine -Placebo	-9.3	13.0

The standard error (SE) of the difference = **1.6**

95% CI

From statistical tables

point estimate ± *(measure of how confident we want to be)* × *(standard error)*

-9.3 ± **1.96** × **1.6** → **(-12.4 to -6.2)**

- We are 95% confident that the true (population) mean difference in SBP comparing amlodipine and placebo is between -12.4 and -6.2.
- Since the 95% CI does not include 0, we conclude that amlodipine is effective in reducing SBP

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Hypothesis testing (1)

- A statistical method that uses sample data to evaluate a hypothesis about a population **parameter**. It is intended to help researchers differentiate between **real** and **random** patterns in the data.

- Hypothesis
  - An assumption about the population parameter.

- The two hypotheses
  - **Null (H_0):** states the assumption to be tested e.g. Amlodipine is not effective in reducing SBP.
  - **Alternative (H_a):** is the opposite of the null hypothesis (Amlodipine reduces SBP). It may or may not be accepted and it is the hypothesis that is believed to be true by the researcher.

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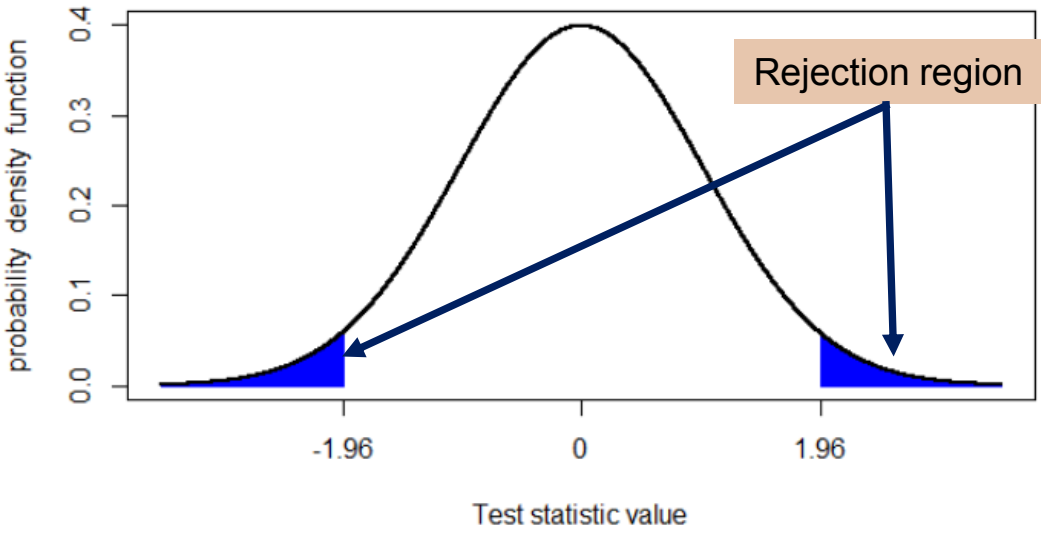
Hypothesis testing (2)

- Level of significance (α), typical values are 0.01, 0.05, 0.1
  - Defines unlikely values of a test statistic if null hypothesis is true. Called rejection region of sampling distribution.



Linked to the p-value test

- If p value ≥ α, Do Not Reject H_0
- If p value < α, Reject H_0 i.e. the test-statistic falls in the rejection region.

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Hypothesis testing - steps

Define your hypotheses (null, alternative)

The null hypothesis is the “straw man” that we are trying to shoot down.

Null: Amlodipine does not reduce systolic blood pressure.

Level of significance: 5%

Specify your sampling distribution (under the null)

If we repeated our experiment many, many times, the mean difference SBP between placebo and amlodipine group is 0.

Calculate the p-value of what you observed

What is the probability of observing a more extreme test statistic?

Make a decision

Reject or **fail to reject** the null hypothesis.

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Hypothesis testing - example

1. Data: Amlodipine vs. Placebo study
2. Hypotheses: H_0: $\mu_A = \mu_P$ $vs.$ H_a: $\mu_A \neq \mu_P$ i.e. mean SBP for amlodipine group is not difference from the mean SBP for the placebo group.
3. Level of significance: 5% or 0.05
4. Test statistic: $t = \frac{\bar{x}_A - \bar{x}_P}{s_{pooled} * \sqrt{\frac{1}{n_A} + \frac{1}{n_P}}}$; s_{pooled} is the pooled standard deviation.

   Thus t = -5.96
5. Distribution of test statistic: Our test statistic is distributed as Student's t with degrees of freedom (df = $n_A + n_P - 2$ = 241) if H_0 is true. Critical value = t(0.975, 241) = 1.96 or -1.96
6. Decision: The computed test statistics value (-5.96) is less than the critical value (-1.96). We reject the null hypothesis. The data provide sufficient evidence to conclude that amlodipine has an effect on SBP. In fact the direction of the test statistic tell us that amlodipine is effective in reducing SBP.

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Fundamentals of biostatistics

Power and sample size calculation

Lesson 9 – Part 2

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Lesson objectives

- Understand why sample-size calculation is important prior to starting a study.
- Understand the components required for sample-size calculation and how to estimate these.
- Define and describe type I and type II error.
- Understand why type I and type II error are a concern to researchers.

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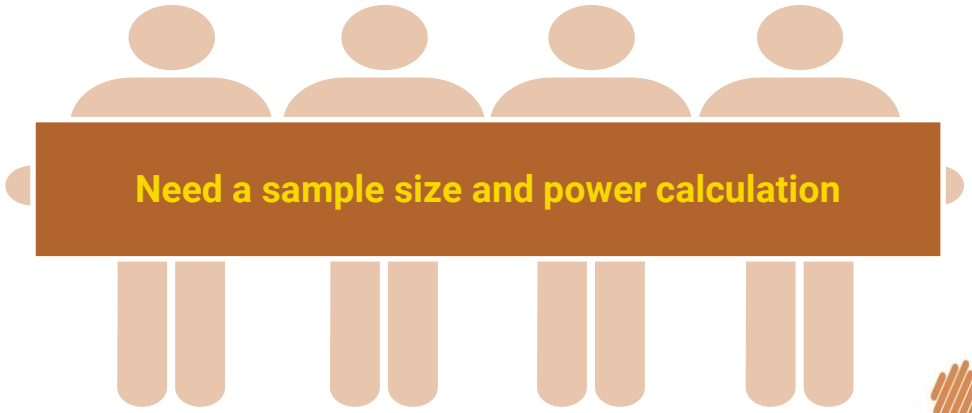
The beginning

We believe we have found a cure for the common cold. How many patients do we need to study to get our product approved by a regulatory authority?



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Why sample size & power

Scientific implications

- Can't be sure we've made right decision regarding the effect of the intervention
- However, we want enough subjects enrolled to adequately address study question to feel comfortable that we've reached correct conclusion. Avoid creating confusion.

Ethical implications

- Too many subjects
  - Wasteful of resources
  - Too many subjects unnecessarily exposed to risk. Should enroll only enough patients to answer study question, to minimize the discomfort and risk subjects may be exposed to.
- Too few subjects
  - Cannot adequately address study question. The time, discomfort and risk to subjects have served no purpose.
  - May conclude no effect of an intervention that is beneficial. Current and future subjects may not benefit from new intervention based on current (inconclusive) study.

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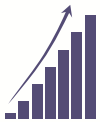
The beginning

Begin with the end in mind

- Visualize the final output and the statistical methods to be used the research question.

- Understand the research question
  - Learn about the application and the problem.
  - Learn about the disease and the medicine.
  - Is the research question **measurable** and will the process produce data that can be supported or contradicted?



- Type of analysis
  - Sample size & power calculations are based upon the planned method of analysis.
  - If you don't know how the data will be analysed (e.g., 2-sample t-test), then you cannot accurately estimate the sample size

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Statistical power – essential concepts

- The null hypothesis H_0
  - States that the findings of the experiment are no different to those that would have been expected to occur by **chance**.

- Type I error & type II error

Decision	H_o is true i.e., there is really no effect to find	H_o is false i.e., there really is an effect to be found
Retain H_o	Correct decision: prob = 1 - α	Type II error: prob = β
Reject H_o	Type I error: prob = α	correct decision: prob = 1 - β

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Statistical power – formal definition

- The probability of not missing an effect, due to **sampling error**, when there really is an effect there to be found.

- Factors influencing statistical power

  - Sample size

  - Level of significance (α)

  - Reasonable minimum effect size

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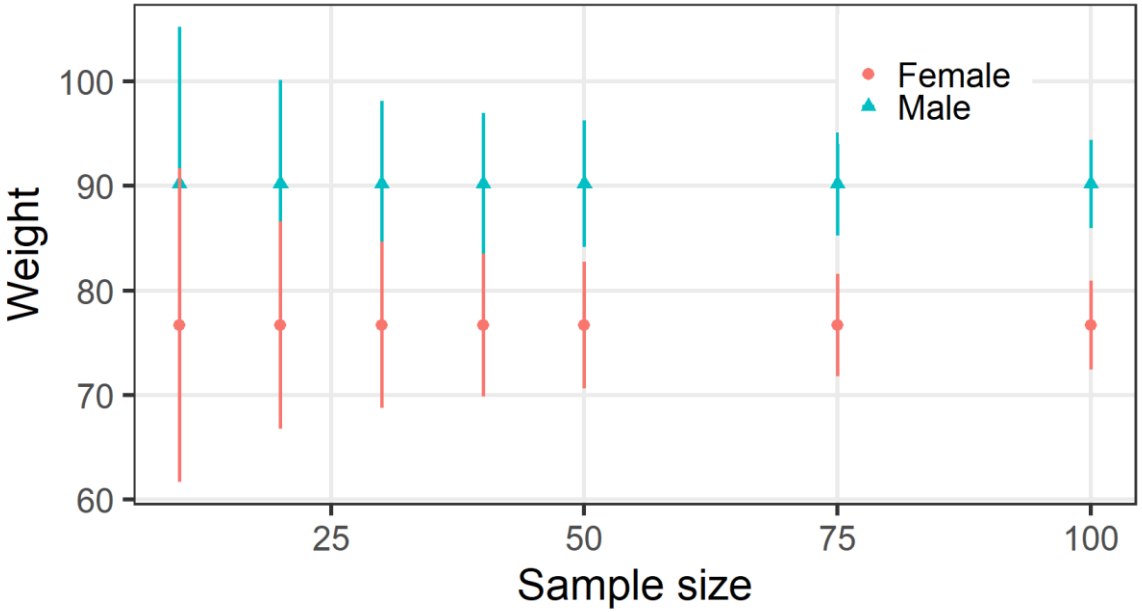
Sample size

One key question in research is: How many subjects we should include in our study?

- All things being equal, the only way to increase the power of an experiment (i.e., increase its chance of detecting true differences) is by increasing the sample size.



Mean (95% confidence interval) weight of males and females

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Sample size calculation - steps

Formulate the study	▪ Define a study design ▪ Choose the outcome summary ▪ Specify the analysis method
Specify analysis parameters	▪ Level of significance ▪ One- or two-sided test ▪ Power of the test
Specify effect size	▪ Expected effect size (“best”) or ▪ Clinically meaningful
Compute sample size or power	▪ Apply specific formulas or use simulations ▪ Use simulations
Sensitivity analysis	▪ Evaluate multiple scenarios to determine relationship between study parameters & sample size or power

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Example - bioequivalence (1)

A researcher would like to evaluate the bioavailability of rifampicin in a four-drug fixed dose combination against a single drug formulation. Each tablet contains 600 mg of rifampicin. Rifampicin concentrations will be measured pre-dose and 1, 2, 3, 4, 6, 8, 12 hours post-dose to compute $AUC_{0\text{-}12}$ using Non-compartmental analysis. How many subjects should be enrolled to be able show that the formulations are bioequivalent?

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Example – bioequivalence (2)

Formulate the study	▪ Define a study design –> **2 period, 2 treatment crossover study** ▪ Choose the outcome summary –> **AUC_{0-12}** ▪ Specify the analysis method --> **Difference between means (natural log)**
Specify analysis parameters	▪ Level of significance –> **5%** ▪ One- or two-sided test –> **two one-sided tests** ▪ Power of the test --> **80%** ▪ True ratio → **0.95** ▪ Coefficient of variation (original scale) → **0.25**
Specify effect size	▪ Expected effect size (“best”) or ▪ Clinically meaningful → **90% CI of the geometric mean ratio of AUC between test and reference should be between 80% and 125%**
Compute sample size or power	▪ Apply specific formulas or use simulations –> **total sample (n = 28)** ▪ Use simulations
Sensitivity analysis	▪ Evaluate multiple scenarios to determine relationship between study parameters & sample size or power → **Vary true ratio or coefficient of variation**

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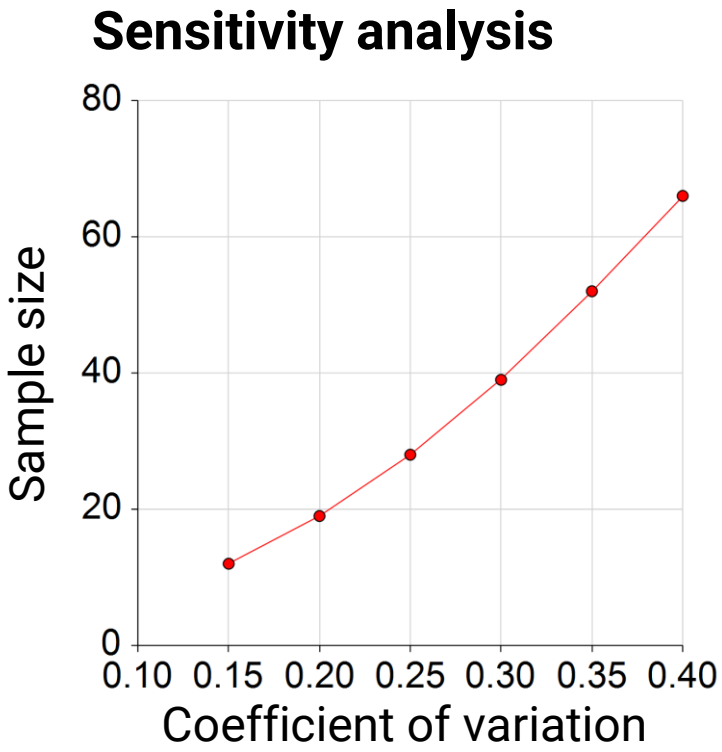
Example - bioequivalence (3)

Summary

For a bioequivalence test using two one-sided tests on data from a two-period cross-over design, a total sample size of 28 achieves 80% power at a 5% significance level when the true ratio of the means is 0.95, the coefficient of variation on the original, unlogged scale is 0.25, and the bioequivalence limits of the mean ratio are 0.8 and 1.25.



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Fundamentals of biostatistics

Types of data and methods of analysis

Lesson 9 – Part 3

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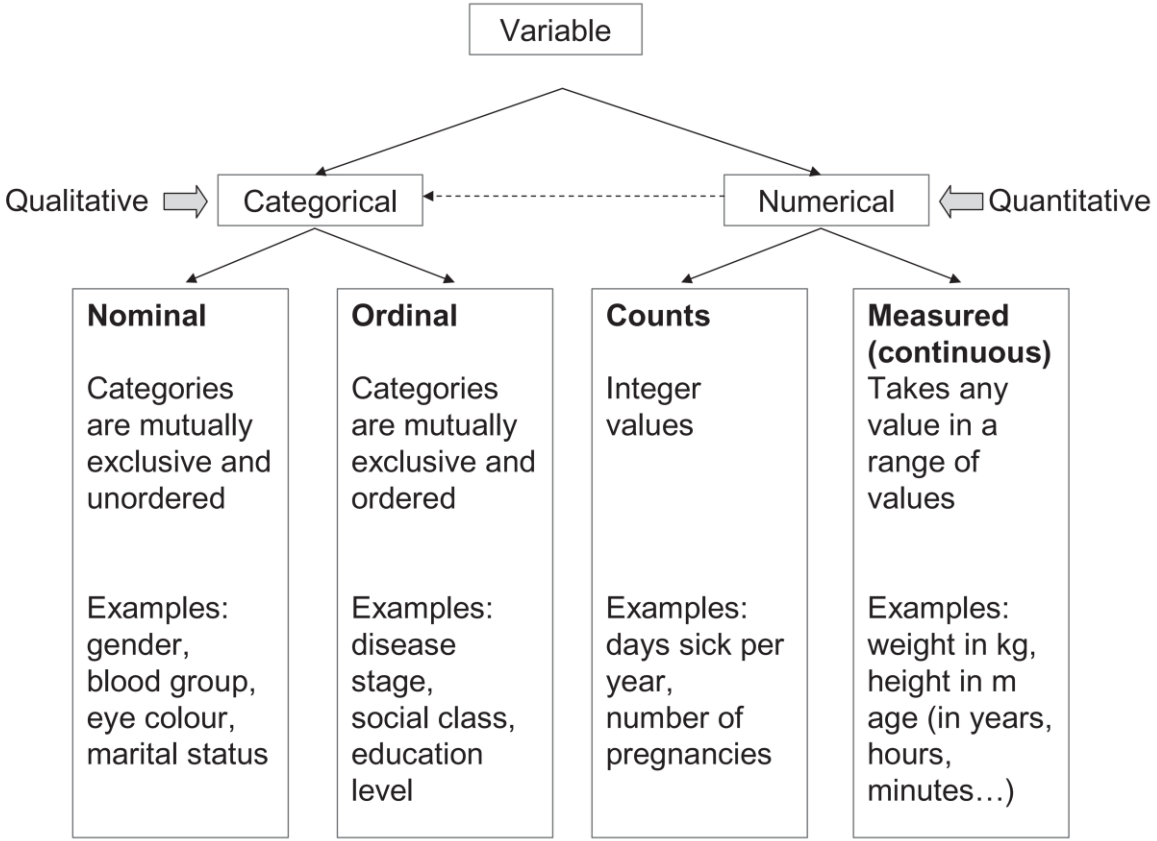
Lesson outcomes

- Recognize and describe types of variables.
- Explore how aspects of the study design impact the statistical analysis.
- Identify the primary statistical tools for various types of studies.
- Perform a simple statistical analysis.

Variable and variable types

Measurable characteristics or features of study subjects that can take on different values.



Source: Machin, D., Campbell M.J., Walters S.J. (2007) *Medical statistics – A text book for health sciences*. West Sussex: John Wiley & Sons Ltd

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Preparing for statistical analyses

Key components

Research question	• Identifies the hypothesis of a statistical test. • Outlines what characteristics will be measured. • Identifies which groups will be compared.
Measurements	• What is to be measured? • How will the characteristic be measured? • Identify dependent and independent variables (where possible).
Study design	• Identify the study population. • Sampling plan e.g No. of sampling time points. • Randomization & treatment allocation.
Analysis method	• Is there an association between characteristics? • Is there a difference between populations (how many)? • Identify a statistical analysis tool appropriate for the study.
Sample size and power	• Specify the level of significance. • Specify the anticipated power of the study based on an effect size. • Compute the sample size for the planned statistical analysis.

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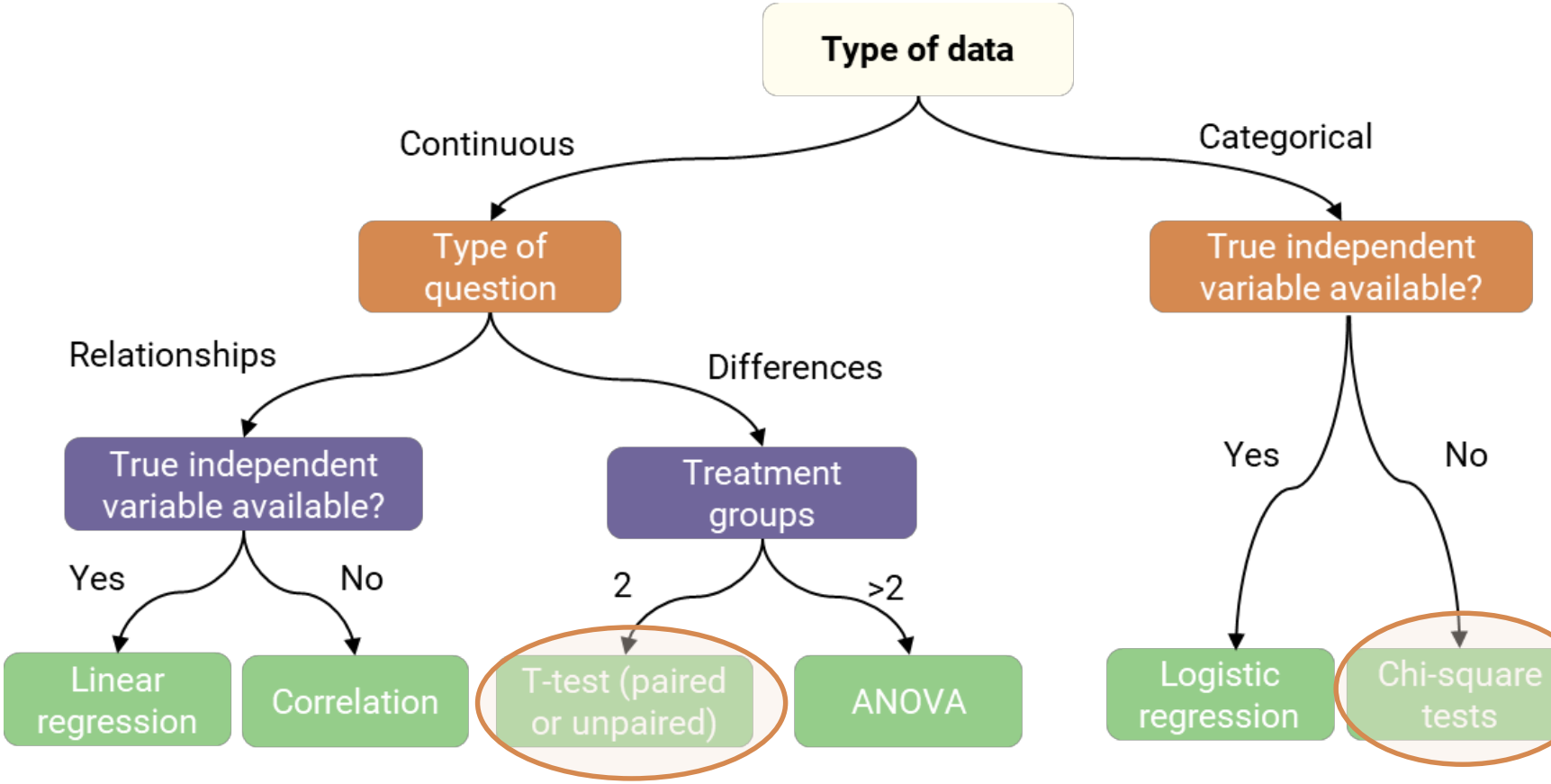
Simple treatment comparisons



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Simple treatment comparison

Two proportions – basic concepts

- Want to compare the responses in two groups or treatments.
- Each sample is considered to be a sample from a distinct population.
- The responses in each group are independent of those in the other group.
- Data are usually presented in a contingency table.

Contingency table setup

Population	Population Proportion	Sample Size	Count of Successes	Sample Proportion
1	p_1	n_1	X_1	$\hat{p}_1 = \frac{X_1}{n_1}$
2	p_2	n_2	X_2	$\hat{p}_2 = \frac{X_2}{n_2}$

- Chi-squared (χ^2) test statistic is widely used in the analysis of contingency tables.
- The null hypothesis for this test is that there is **no association** between the variables.
- A significant p-value implies association.

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Simple treatment comparison

Two proportions – example

- Rates of microbiological cure according to number of Grade 3 or 4 adverse events experienced by patients taking standard TB therapy.

	No. of grade 3/4 AEs	Microbiological cure	No microbiological cure	Total
Total	0	464 (90.8%)	47 (9.2%)	511
	≥1	101 (78.9%)	27 (21.1%)	128
Related only	0	523 (89.9%)	59 (10.1%)	582
	≥1	42 (73.7%)	15 (26.3%)	57

Tweed CD, et al. Toxicity associated with tuberculosis chemotherapy in the REMoxTB study. BMC Infect. Dis. 2018;18(1):317.

- Patients are grouped by the number of AEs they experienced in the trial.

- The number of patients who were either cured or not cured of their TB are displayed with row percentages (**Chi-square test p value < 0.001**) in two groups or treatment.

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Simple treatment comparison

Two means

- It is more common to want to investigate how two means differ from one another – or if they differ at all.

- We can compare the means from
  i. two separate groups ("independent samples"), or
  ii. two sets of observations taken on the same group ("paired data").

- Paired data is common in bioequivalence studies.

  - The objective is to test the hypotheses

$$H_0: Bioinequivalence$$
$$\text{vs}$$
$$H_a: Bioequivalence$$

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Simple treatment comparison

Two means – bioequivalence example

- PK data were collected from 32 subjects; 17 received the formulations in the reference-test (RT) and 15 in the test-reference (TR) order.
- The main reason for using a cross-over design is to make comparisons between the two formulations 'within' each subject and as a result to eliminate any between-subject variability.

Subject	1	4	5	8	9	11	16	17	19	21	24	25	28	29	31	34	36
Period 1	2849	2790	2112	1736	1356	1775	2997	1973	1454	2469	1584	4004	1944	1175	1696	1737	2040
Period 2	2230	2864	1744	1882	1175	1585	2237	1778	1297	2023	1855	2449	1593	1147	1801	1655	2199
Sequence	RT	RT	RT	RT	RT	RT	RT	RT	RT	RT	RT	RT	RT	RT	RT	RT	RT
Subject	2	3	6	7	10	12	15	18	20	22	23	26	27	30	35		
Period 1	2025	2090	2006	2202	1838	1898	1129	2014	1900	1763	1678	2271	1986	2519	1560		
Period 2	2000	1826	1881	1935	1602	2504	1036	1938	1730	1472	1336	2389	1857	1941	1629		
Sequence	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR	TR		

- There is need to have a closer look at the data e.g using graphical techniques

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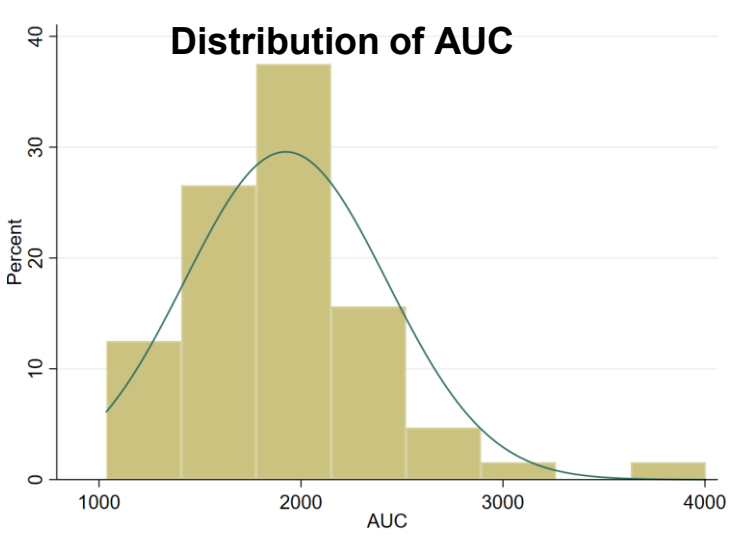
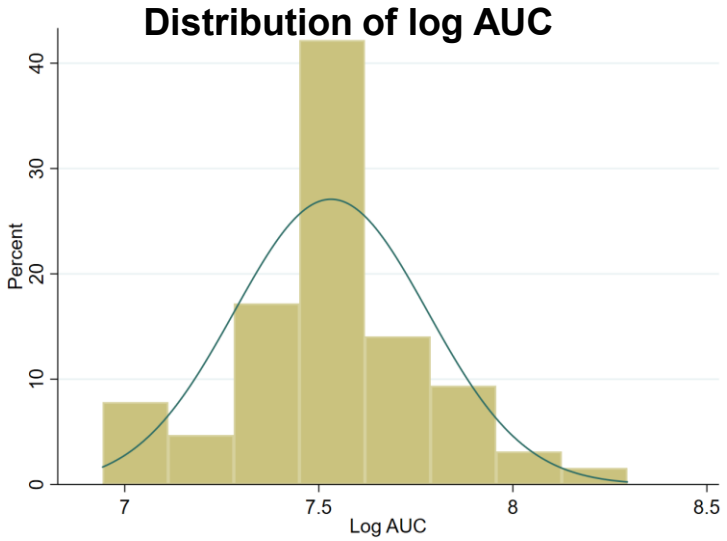
Simple treatment comparison

Two means – bioequivalence example





- It is clear that the distribution of $\log_e$ -transformed AUC is more symmetric → we can apply statistical tools involving the normal distribution.
- To test whether the two formulations are bioequivalent, we will use confidence interval approach.

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Simple treatment comparison

Two means – bioequivalence example

Summary of the log transformed data

Sequence	Period 1	Period 2	Mean
RT	7.60	7.50	7.55
TR	7.55	7.48	7.51
Mean	**7.58**	**7.49**	**7.53**

The mean difference is a weighted average of the difference between periods within each sequence, starting with the TR sequence.

$$Mean\ difference = \frac{1}{2}\{(7.55 - 7.48) - (7.60 - 7.50)\}$$
$$= -0.0166$$

- The standard error of the difference is a weighted average of the within-subject variance.
- The value is **0.0263**
- The 90% confidence interval is - 0.0612 to 0.028, on the log scale.
- On the normal scale the 90% CI: **0.94 to 1.03 → Two formulations are bioequivalent.**

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Simple treatment comparison

Analysis of variance (ANOVA)

- ANOVA is an extension of the t-test for independent groups, allows comparison of more than 2 groups.
- ANOVA compares the variance between groups with the variance within groups to identify if group means are different.
- The **null hypothesis** is: there is no difference among groups (e.g. 3 groups).

$$\mu_A = \mu_B = \mu_C$$

- **Alternative hypothesis**: at least one pairing is different.
- If one pairing is different further tests are required to identify the pairing. Similar to a set of t-tests but controlling for the level of significance.

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Advanced statistical methods

- Population pharmacokinetics and pharmacodynamics
- Time to event analysis / Survival analysis
- Analysis of clustered data

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