# P8130: Biostatistical Methods I

## Lecture 7: Methods of Inference for Two-Means

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# Lecture 6: Recap

- Sampling Distribution
- Central Limit Theorem (additional simulations in Recitation 2)
- Estimation/Confidence Interval for One-Sample Mean
- Hypothesis Testing for One-Sample Mean

# Lecture 7: Outline

- Inference for two-samples means equal and unequal variances:
  - Confidence interval
  - Hypothesis testing
  - Test for equality of variances

# Two-Sample Inference

- Thus far we were only interested in one parameter from a single population (one sample)
- What if we want to compare parameters in more than one population?
- In a two-sample setting, we want to make inferences about the parameters of two populations (both values unknown)
  - Paired vs Independent <-> Longitudinal vs Cross-sectional study

# The Paired t-test

<u>Example:</u> You are testing a new blood pressure medication on a sample of *n* randomly selected patients that came for a regular consult at CUMC in 2016. Systolic blood pressure (SBP) measurements were recorded at the first visit and 6 months later for all subjects.

<u>Data structure: paired samples</u>

| Visit 1 | | Visit 2 | Differences |
|---|---|---|---|
| $X_{11}$ | $\rightarrow$ | $X_{12}$ | $d_1 = X_{11} - X_{12}$ |
| $X_{21}$ | $\rightarrow$ | $X_{22}$ | $d_2 = X_{21} - X_{22}$ |
| $\vdots$ | | $\vdots$ | $\vdots$ |
| $X_{n1}$ | $\rightarrow$ | $X_{n2}$ | $d_n = X_{n1} - X_{n2}$ |

# The Paired t-test

<u>Assumptions:</u>

SBP measurements are normally distributed with mean $\mu_1$ and $\mu_2$, for visit 1 and 2, respectively.
If follows that the differences $d_i \sim N(\Delta, \sigma_d^2), i = 1,2, \dots, n$.

| Visit 1 | | Visit 2 | Differences |
|---|---|---|---|
| $X_{11}$ | $\rightarrow$ | $X_{12}$ | $d_1 = X_{11} - X_{12}$ |
| $X_{21}$ | $\rightarrow$ | $X_{22}$ | $d_2 = X_{21} - X_{22}$ |
| | | $\vdots$ | $\vdots$ |
| $X_{n1}$ | $\rightarrow$ | $X_{n2}$ | $d_n = X_{n1} - X_{n2}$ |

The problem reduces to *one-sample t-test* based on the differences $d_i$ (variance unknown), where:

$$\bar{d} = \sum_{i=1}^{n} d_i / n \text{ and } s_d = \sqrt{\sum_{i=1}^{n} \left(d_i - \bar{d}\right)^2 / (n-1)}$$, n represents # of pairs

# Two-Sided Paired t-test

<u>Test for the mean of the differences with unknown variance</u>

$$H_0\colon \mu_1 - \mu_2 = 0 \; or \; \Delta = 0$$

vs.

$$H_1\colon \mu_1 - \mu_2 \neq 0 \; or \; \Delta \neq 0$$

With significance level α pre-specified, compute the test statistic:

$$t = \frac{\bar{d} - 0}{s_d/\sqrt{n}}$$

Reject $H_0$: if $|t| > t_{n-1,1-\alpha/2}$
Fail to reject $H_0$: if $|t| \leq t_{n-1,1-\alpha/2}$

$t_{n-1,1-\alpha/2}$ is called the critical value and it can be found in tables or calculated using software.

# Confidence Interval for Δ

A $100(1-\alpha)\%$ confidence interval for the true mean difference (Δ) for two paired samples is given by:

$$\bar{d} - t_{n-1,1-\alpha/2}\frac{s_d}{\sqrt{n}} \leq \Delta \leq \bar{d} + t_{n-1,1-\alpha/2}\frac{s_d}{\sqrt{n}}$$

Where:

$\bar{d}$ is the point estimate of the mean difference

$\frac{s_d}{\sqrt{n}}$ is the estimated standard error of the differences

$t_{n-1,1-\alpha/2}$ is the percentile of the *t*-distribution with (n-1) degrees of freedom

# The Paired t-test: Example

Test if a new diet has an effect on lowering the cholesterol levels. Use the data below recorded from 12 randomly selected subjects (time interval: 3mo).

| Subject | Before ($X_1$) | After ($X_2$) | Difference (After-Before) |
|---|---|---|---|
| 1 | 201 | 200 | -1 |
| 2 | 231 | 236 | 5 |
| 3 | 221 | 216 | -5 |
| 4 | 260 | 233 | -27 |
| 5 | 228 | 224 | -4 |
| 6 | 237 | 216 | -21 |
| 7 | 326 | 296 | -30 |
| 8 | 235 | 195 | -40 |
| 9 | 240 | 207 | -33 |
| 10 | 267 | 247 | -20 |
| 11 | 284 | 210 | -74 |
| 12 | 201 | 209 | 8 |

# The Paired t-test: Example

Assume the observed differences constitute a random sample from a normally distributed population of differences.

In class practice.

# Two-Sample t-test for Independent Samples

- Consider that our two samples are independent (there is no relation / correlation between the data points from the two groups) and that they are normally distributed:

$$X_1 \sim N(\mu_1, \sigma_1^2) \text{ and } X_2 \sim N(\mu_2, \sigma_2^2)$$

We can assume that the underlying variances of the two samples are <u>equal</u>:

$$\sigma_1^2 = \sigma_2^2 = \sigma^2$$

We can assume that the underlying variances of the two samples are <u>unequal</u>:

$$\sigma_1^2 \neq \sigma_2^2$$

# Two-Sample Independent t-test: Equal Variances

- If we know the two population variances, then:

$$\overline{X_1} - \overline{X_2} \sim N\left(\mu_1 - \mu_2, \frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}\right)$$

- Because $\sigma_1^2 = \sigma_2^2 = \sigma^2$, it follows that:

$$\overline{X_1} - \overline{X_2} \sim N\left(\mu_1 - \mu_2, \frac{\sigma^2}{n_1} + \frac{\sigma^2}{n_2}\right)$$

- Thus, testing $H_0: \mu_1 = \mu_2$ can use the test statistic:

$$z = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\sigma^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

# Two-Sample Independent t-test: Equal Variances

- However, most of the times we do not know the common variance and we have to find <u>one</u> estimator using the sample variances $s_1^2$ and $s_2^2$.

- The pooled estimate of the variance from two independent samples is given by:

$$s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \quad (*)$$

- Explain the denominator.

# Two-Sample Independent t-test: Equal Variances

$H_0$: $\mu_1 = \mu_2$ vs $H_1$: $\mu_1 \neq \mu_2$ (two-sided)

With significance level α pre-specified, compute the test statistic:

$$t = \frac{\overline{X_1} - \overline{X_2}}{s\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$, where $s$ is given in (*).

Reject $H_0$: if $|t| > t_{n_1+n_2-2,1-\alpha/2}$
Fail to reject $H_0$: if $|t| \leq t_{n_1+n_2-2,1-\alpha/2}$

P-value $= 2 \times \mathrm{P}(t_{n_1+n_2-2} < t)$, if $t < 0$
$= 2 \times \mathrm{P}(t_{n_1+n_2-2} \geq t)$, if $t \geq 0$

# Two-Sample Independent t-test: Unequal Variances

- If we know the two population variances, then:

$$\overline{X_1} - \overline{X_2} \sim N\left(\mu_1 - \mu_2, \frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}\right)$$

- For testing $H_0: \mu_1 = \mu_2$ we can use the z-test statistic:

$$z = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\left(\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}\right)}}$$

- Or (again) use $s_1^2$ and $s_2^2$ to estimate the two population variances (if these values are unknown).

# Two-Sample Independent t-test: Unequal Variances

$H_0$: $\mu_1 = \mu_2$ vs $H_1$: $\mu_1 \neq \mu_2$ (two-sided)

With significance level α pre-specified, compute the test statistic:

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

Compute the approximating degrees of freedom $d'$, and round down to the nearest integer $d''$:

$$d' = \frac{\left(\frac{s_1^2}{n_1}+\frac{s_2^2}{n_2}\right)^2}{\left(\frac{s_1^2}{n_1}\right)^2/(n_1-1)+\left(\frac{s_2^2}{n_2}\right)^2/(n_2-1)} \quad (**)$$

# Two-Sample Independent t-test: Unequal Variances

$H_0$: $\mu_1 = \mu_2$ vs $H_1$: $\mu_1 \neq \mu_2$ (two-sided)

With significance level α pre-specified, compute the test statistic:

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

Reject $H_0$: if $|t| > t_{d'',1-\alpha/2}$

Fail to reject $H_0$: if $|t| \leq t_{d'',1-\alpha/2}$

Where $d''$ represents the nearest integer of $d'$ (degrees of freedom) $(**)$.

# Confidence Intervals for Two Independent Samples

Two-sided $100(1-\alpha)\%$ confidence interval for:

- Two-independent samples (equal variance): $\mu_1 - \mu_2(\sigma_1^2 = \sigma_2^2 = \sigma^2)$:

$$(\overline{X_1} - \overline{X_2} - t_{n_1+n_2-2,1-\alpha/2} s\sqrt{1/n_1 + 1/n_2}, \overline{X_1} - \overline{X_2} + t_{n_1+n_2-2,1-\alpha/2} s\sqrt{1/n_1 + 1/n_2})$$

- Two-independent samples (unequal variance): $\mu_1 - \mu_2(\sigma_1^2 \neq \sigma_2^2)$:

$$(\overline{X_1} - \overline{X_2} - t_{d'',1-\alpha/2}\sqrt{s_1^2/n_1 + s_2^2/n_2}, \overline{X_1} - \overline{X_2} + t_{d'',1-\alpha/2}\sqrt{s_1^2/n_1 + s_2^2/n_2})$$

Where $d''$ represents the nearest integer of $d'$ (degrees of freedom) $(**)$.

# Test for Equality of Variances

Assume two independent random samples from $N(\mu_1, \sigma_1^2)$ and $N(\mu_2, \sigma_2^2)$.

Testing the equality of variances implies testing the hypotheses:

$$H_0: \sigma_1^2 = \sigma_2^2 \text{ vs } H_1: \sigma_1^2 \neq \sigma_2^2$$

The significance test is based on the relative magnitudes of the sample variances. (Why?)
With significance level α pre-specified, compute the test statistic:

$$F = \frac{s_1^2}{s_2^2} \sim F_{n_1-1, n_2-1}$$

The test statistic follows an F distribution with $n_1 - 1$ and $n_2 - 1$ degrees of freedom.

# Test for Equality of Variances

Testing the hypotheses:

$$H_0: \sigma_1^2 = \sigma_2^2 \text{ vs } H_1: \sigma_1^2 \neq \sigma_2^2$$

With significance level α pre-specified, compute the test statistic:

$$F = \frac{s_1^2}{s_2^2} \sim F_{n_1-1,n_2-1}$$

Reject $H_0$: if $F > F_{n_1-1,n_2-1,1-\alpha/2}$ or $F < F_{n_1-1,n_2-1,\alpha/2}$

Fail to reject $H_0$: if $F_{n_1-1,n_2-1,\alpha/2} \leq F \leq F_{n_1-1,n_2-1,1-\alpha/2}$

P-value $= 2 \times \mathrm{P}(F_{n_1-1,n_2-1} > F)$, if $F \geq 1$
$= 2 \times \mathrm{P}(F_{n_1-1,n_2-1} < F)$, if $F < 1$

# Two-Samples Independent t-test

Example: To assess the impact of oral contraceptive use on bone mineral density (BMD), researchers in Canada carried out a study comparing BMD for women who had used oral contraceptives (OC) for at least 3 months to BMD for women who had never used oral contraceptives (OC). Summary values for BMD are given below:

| | n | $\bar{X}$ | s |
|---|---|---|---|
| Non OC users | 10 | 1.08 | 0.16 |
| OC users | 10 | 1.00 | 0.14 |

a) Assuming that the BMD is normally distributed in both OC and non-OC users, is there enough evidence to conclude that BMD levels differ between the two groups?
b) Construct a 95% CI for the population mean difference.

In class practice.

# Readings

Rosner, *Fundamentals of Biostatistics*, Chapter 8

- Sections: 8.1 – 8.7