

48th ICRO-SUN PG TEACHING PROGRAMME



CLINICAL TRIAL & CANCER STATISTICS

Generating hypothesis for an oncology study and hypothesis testing

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Outline

- Research Question
- Objective
- Hypothesis Generation
- Hypothesis Testing
- Different statistical Test
- Questions

Background

- Critical step is to ask questions
 - preventive, diagnostic, or therapeutic approaches



A research question is a **specific, directed inquiry** within **lacunae of existing body of knowledge**, which the researcher then **seeks to answer.**

Figure: Process for achieving a good research question

Research Question: FINER



Hulley S, Cummings S, Browner W, et al. Designing clinical research. 3rd ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2007.

Research Question: PICOT



*Not all of these elements need to be present in all research questions

PICOT

- Population: Specific
 - Inclusion and Exclusion Criteria: Age, Gender, Co-morbidities, type of tumor
 - Eg: To identify circulating microRNAs able to identify **ovarian cancer patients at high risk for relapse**
- Intervention/Exposure
 - Treatment, procedure, therapy, or placebo
 - How to measure: clinical outcomes, surrogates (biomarkers), questionnaires, quality-of-life scales, cost effectiveness
 - Eg: evaluate the security and effectiveness of <u>cisplatin with constant dose intense</u> <u>temozolomide (TMZ)</u> for reduplicative glioblastoma multiforme (GBM) within 6 months

PICOT

- Comparison: Similar to experimental group except active treatment
 - Placebo, standard care or practice, a different therapy
 - Eg: To investigate the clinical efficacy of <u>Vandetanib plus gemcitabine versus placebo</u> <u>plus gemcitabine</u> in locally advanced or metastatic pancreatic adenocarcinoma
- Outcome
 - Objective response rate, progression-free survival, overall survival, and patient-reported outcomes
 - Eg: Evaluate whether antiandrogen therapy plus radiotherapy would further improve cancer control as a salvage therapy for recurrent localized prostate cancer and **prolong overall survival** in comparison with radiation therapy alone

PICOT

- Outcome
 - Primary
 - Secondary
- Time
 - Duration of the data collection and the follow-up period for the main event
 - Depend on type of cancer and its prognosis
 - Eg: Compare Intensity-modulated radiotherapy with or without weekly cisplatin for the treatment of locally recurrent nasopharyngeal carcinoma between April 2022 and January 2022

Examples of research question

- Is drug ABC more efficient than drug XYZ in patients with cervical cancer? (Bad RQ)
- Is drug ABC more efficient in alleviating distress than drug XYZ in patients with cervical cancer? (Bad RQ)
- Is drug ABC more efficient in alleviating distress than drug XYZ in patients with cervical cancer within 2 years? (Good RQ)
- $P \rightarrow Patients$ with cervical cancer
- $\mathbf{I} \rightarrow \mathbf{Drug} \mathbf{ABC}$
- $\mathbf{C} \rightarrow \mathbf{Drug} \ \mathbf{XYZ}$
- $\mathbf{O} \rightarrow \mathbf{Distress}$
- $T \rightarrow 2$ years

Aim and objectives:

Aim: Ultimate, broad, long-term goal or outcome the researcher wants to achieve

Objective: specific, short-term outcomes that the researcher wants to achieve by continuously chasing it.

- Primary objective: identifies a clear focus and helps determine the sample size and feasibility of the study
- **Secondary objective**: Additional goals or outcomes that support the primary objectives and may be associated factors

Objectives: SMART

Objectives Checklist

Criteria to assess objectives			NO
1.	 Is the objective SMART? Specific: Who? (target population and persons doing the activity) and What? (action/activity) Measurable: How much change is expected Achievable: Can be realistically accomplished given current resources and constraints Realistic: Addresses the scope of the health program and proposes reasonable programmatic steps Time-phased: Provides a timeline indicating when the objective will be met 		
2.	Does it relate to a single result?		
3.	Is it clearly written?		

Randomized controlled trial to evaluate the effects of combined progressive exercise on metabolic syndrome in breast cancer survivors: rationale, design, and methods

- **Research Question**: What is the effect of combined (aerobic and resistance) exercise on components of MetS, as well as on physical fitness and QOL, in breast cancer survivors
- Aim: To assess the effects of combined (aerobic and resistance) exercise on components of MetS, as well as on physical fitness and QOL, in breast cancer survivors soon after completing cancer-related treatments.
 - Primary objective: To determine the effects on Components of metabolic syndrome (MetS)
 - Waist circumference, blood pressure, fasting glucose, HDL, triglycerides
 - Secondary objectives: To determine effects on
 - cardiorespiratory fitness, muscle strength, body composition, quality of life, shoulder strength and range of motion (ROM), and serum levels of insulin, C-reactive protein, and glycosylated hemoglobin (HbA1c).

Hypothesis

- Clear statement of the expected research outcomes
- Not needed in descriptive studies
 - Declarative form (not interrogative)
 - Identifies PICO or PIO
 - Relationship between two variables
 - Testable
 - Operational
 - States the expected outcome of study

Patients with primary glioblastoma that undergo **surgery followed by adjuvant radiotherapy and chemotherapy** have **longer survival** than patients receiving only **adjuvant radiotherapy**

Hypotheses: Null vs Alternate

Research Question

Is there a difference between two groups with respect to the outcome?

• Is drug is better than the standard one for treating a certain type of tumor

Null Hypothesis

There is no difference between two groups with respect to the outcome.

• H0: The new drug has a similar (not better and not worse) frequency of adverse effects compared with the standard one for treating castrate-resistant prostate cancer.

Alternative Hypothesis

There is a (statistically) significant difference between two groups with respect to the outcome.

- **Ha/1:** The new drug has a lower frequency of adverse effects than the standard one for treating prostate cancer.
- or
- Ha: The new drug has a greater frequency of adverse effects than the standard one for treating prostate cancer.

Hypotheses: Directional vs Non directional

- Smoking is not associated with lung cancer (Null hypothesis)
- **Directional** : foretell the **direction of relationship** between independent and dependent variables
 - Smoking is associated with a higher incidence of lung cancer (One-tailed hypothesis) or
 - Smoking is associated with a lower incidence of lung cancer (or it is protective) (Onetailed hypothesis)
- Non directional
 - Smoking has some association with lung cancer (uncertain of how it influences lung cancer) (**Two-tailed hypothesis**)

Types of Hypotheses

- Null Hypothesis (H₀): no difference in outcome between the treatments
- Alternative Hypothesis (H₁): difference in outcome between the treatments
- Example: Difference in means

 $\begin{array}{ll} H_0: \ \mu_1 = \ \mu_2 \\ against \\ H_1: \ \mu_1 < \mu_2 \\ (Left tailed) \end{array} \begin{array}{ll} H_1: \ \mu_1 \neq \mu_2 \\ (two-tailed) \end{array} \begin{array}{ll} H_1: \ \mu_1 > \mu_2 \\ (two-tailed) \end{array}$

Errors related to hypothesis- the Court Judgment Model

- **Presumption of innocence** the convict is innocent unless proven guilty.
 - This assertion is analogous to H0
- Burden of proof of guilt of the convict lies with the investigator
- We reject presumption of innocence convict is proved to be not innocent
 - This assertion is analogous to H1
- In this case there are four possibilities:

Court Judgement Actual Position	Pronounced guilty	Pronounced not guilty
Crime not committed	Serious error	Correct decision
Crime committed	Correct decision	Less serious error

Errors related to hypothesis

Possibilities	H0 rejected, H1 accepted	H0 not rejected, H1 rejected
Null hypothesis true/no difference exists in real life	Wrong decision– Type I error (α)	Correct decision– Level of confidence (1-α)
Null hypothesis is untrue/a difference exists in real life	Correct decision–Power of the study (1-ß)	Wrong decision Type II error (ß)

• Type I error is more grave than Type II error

Types of errors

• **Type I error**: finds difference in treatments when in actuality no difference

 $P(Type \ I \ error) = \alpha$

• **Type II error**: fails to find a difference in treatments when in actuality there is a difference

P(Type II error) = β

Errors related to hypothesis: Summary

- α: Probability of making a **Type I error**
 - Probability of rejecting null hypothesis (hypothesis of no difference) when it is in fact true
 - Usually kept at 5% (or 0.05 if expressed in decimal).
- **1-α**: Probability that our study will correctly rule out a difference if in reality no difference exists
 - Known as **level of confidence**
- **B**: Probability of making a Type II error
 - Probability that our study will fail to find an association if in reality, an association exists.
 - Usually set at 20%. (0.2 in decimal)
- **1-B:** Probability that our study will correctly find an association if in reality an association exists (**power**)

Confidence Limit

			•	Confidence limit or confidence interval is the
				upper and lower limit (range) of a parameter
Fig	gure 1			(eg, mean) for a given level of confidence
			•	It depends on the pre decided value of α
			•	Interpretation: In the population, 95% values
				fall within the non-shaded area (confidence
				limits: -1.96 to +1.96SD)
			•	Conversely we can say: any extreme values
$\alpha/2$	2		$\alpha/2$	falling beyond the confidence limits (shaded
				area) is rare occurrence (only 5% probability of
				occurring)
-2.58 S	D -1.96SD	Population mean	1.96SD 2.58 SD	If α were to be changed to 1%, 99% values
	•			would fall within the non-shaded area
	9	5% confidence limit	•	As α decreases, the confidence interval
•	99%	confidence limit	>	increases.

Statistical Significance



- Hence, if a sample mean lies *within the shaded area*, it is an extreme value that is *significantly different* from 95% of population values. **The null hypothesis is rejected**.
- If the sample mean lies on the *border* or within the *non-shaded area*, it falls within the range of 95% population (confidence interval). **The null hypothesis is not rejected**.
- This cut-off for maximum allowable error (α) is also known as level of significance.
- Statistical significance is decided by p-value which is computed while testing the hypothesis.
- p-value is the probability that the results we have obtained after hypothesis testing are purely by chance.
- If $p < \alpha$, our results are statistically significant
- If $p > \alpha$, our results are statistically insignificant.

Level of Significance, a and the Rejection Region



Interpreting the p-value...



The critical value of z for $\alpha = 0.05$ is z = 1.645 (i.e., 5% of the distribution is above 1.645) The critical value of z for $\alpha = 0.025$ is z = 1.96 (i.e., 2.5% of the distribution is above 1.96)

P-value

- Probability of obtaining result by chance rather than as a true effect
- Probability of obtaining a test statistics (≤ or ≥) more extreme than actual sample value given H₀ is true
- Observed level of significance
- *P* value $< \alpha$ indicates significant results
 - where α is the maximum permissible Type I error- 5%
 - P > 0.05 -Do not reject H₀ /fails to reject
 - Results are statistically insignificant.
 - P < 0.05 reject H₀
 - Results are statistically significant

Analyses

- Univariate (one variable at a time)
- Bivariate (two variables at a time)
- Multivariate (more than two variables at a time)

Univariate Analysis

Categorical

Frequency percentage prevalence and Incidence

Quantitative

Mean Median Range/IQ Range SD

Bivariate Analyses

- 1. Categorical vs Categorical
- 2. Categorical vs Quantitative
- 3. Quantitative vs Quantitative

Points to consider in choosing the appropriate Statistical test

- 1. Combination of two variables
- 2. Normal or Non-normal
- 3. Groups 2 or >2
- 4. Related or non-related

Skewed Distribution





Tests of Normality

	Kolmogorov–Smirnov ^a			Shapiro–\		c
	Statistic	df	Sig.	Statistic	df	Sig.
Birth weight	0.067	139	0.200*	0.981	139	0.056
Gestational age	0.151	133	0.000	0.951	133	0.000
Length of stay	0.241	132	0.000	0.643	132	0.000

*This is a lower bound of the true significance.

^aLilliefors significance correction.





Figure 2.1 Pathway for the analysis of continuous variables.

1. Categorical vs Categorical



X :Group variable

Y :Outcome variable

Categorical vs Quantitative

Parametric

X=2 & Y: Normal

UnrelatedRelatedStudent's t testPaired 't' test

<u>X>2 & Y: Normal</u>

Unrelated	Related
One way	Repeated
ANOVA	measures ANOVA

Categorical vs Quantitative

Parame	tric	Non-Parametric			
<u>X=2 &</u>	Y: Normal	<u>X=2 & Y:</u>	Non Normal		
Unrelated Student's t test	<i>Related</i> Paired 't' test	<i>Unrelated</i> Wilcoxon ranksum	Related Wilcoxon signrank		
<u>X>2 &</u>	Y: Normal	<u>X>2 & Y</u>	: Non-Normal		
<i>Unrelated</i> One way ANOVA	<i>Related</i> Repeated measures ANOVA	Unrelated <mark>Kruskal Wallis</mark>	<i>Related</i> Freidmans test		

Steps of hypothesis testing

- **1.** State the Hypotheses in terms of population parameters
 - *Ho* Null hypothesis, usually the opposite of our research hypothesis
 - Ha Alternative hypothesis, corresponds to our research hypothesis
- 2. Fixation of the **level of significance**
 - α , Type I error, 5% risk of being in error when rejecting Ho asserting that groups differ
- 3. Selection of the **appropriate test to be utilized**
- 4. Calculating the **critical ratio**
- 5. Comparing the calculated value with the table value (if Critical Value >Test Value, p <0.05, statistically significant)
- 6. Making inferences
 - If P-value $>\alpha$ then fail to reject the null hypothesis
 - "There is insufficient evidence to conclude [*Ha* in words]
 - If P-value $< \alpha$ then reject the null hypothesis.
 - "There is sufficient evidence to conclude [*Ha* in words]

Steps of hypothesis testing

- Decision rule: circumstances to reject the null hypothesis for a specific test depends on
 - Research hypothesis
 - Test statistics: based on distribution z, t
 - Decision rule is based on standard normal distribution (z) or t distribution
 - Level of significance: if α is 0.05, critical value is 1.645
 - Whether an upper-tailed, lower-tailed, or two-tailed test is proposed
 - Upper tailed test reject H if the test statistic is greater than or equal to the critical value
 - Lower-tailed test reject H if the test statistic is less than or equal to the critical value
 - In a two-tailed test, reject H if the test statistic is extreme—either greater than or equal to an upper critical value or less than or equal to a lower critical value





Rejection Region for Lower-Tailed Z Test (H1 : $\mu < \mu$) with $\alpha = 0.05$

Rejection Region for Upper-Tailed Z Test (H1 : $\mu > \mu$) with $\alpha = 0.05$



Rejection Region for Two-Tailed Z Test (H1 : $\mu \neq \mu$) with $\alpha = 0.05$



Contingency Table

Exposure Total Yes No b а (n₁₁) (n₁₂) Exposed a + b d С c + d (n₂₁) (n₂₂) Unexposed a + c n **b** + d

Outcome

Most common way to summarize categorical data

Comparing two proportion

Is particular medicine more effective than another?

Researcher would be interested in studies involving comparison of groups, say, R_x A vs. R_x B.

- Chance variation
- Effect variation

OUTCOME: Cure \odot / Not Cure \otimes

Proportion Test

	CURE	NO CURE	TOTAL
R _x A	20 (5.1%)	373 (94.9%)	393
R _x B	6 (1.9%)	316 (98.1%)	322

What is our interest?

R_x A cure rate Vs **R**_x B cure rate

Comparing two proportion

Null hypothesis:

The hypothesis of "no difference" or no effect" in the population is called null hypothesis.

Cure rate in R_x A group = Cure rate in R_x B group Research (or) Alternative hypothesis:

Research hypothesis states that there is difference.

Treatment A \neq Treatment B

Procedure & Steps

- Find the type of problem and the question to be answered
- State the Null Hypothesis
- State the research Hypothesis
- Selection of the appropriate test to be utilized
- Fixation of the level of significance
- Calculating the critical ratio

Difference in the proportions Standard Error

- Comparing the calculated value with the table value (CV>TV \longrightarrow p<0.05 significant).
- Making inferences.

Comparing two proportion Proportion of people cured in A (p1)= $x_1/n_1 = 20/393 = 0.051$

Proportion of people cured in B (p2)= $x_2/n_2 = 6/322 = 0.019$

$$z = \frac{|\mathbf{p}_1 - \mathbf{p}_2|}{\sqrt{\mathbf{PQ}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \qquad \qquad \mathbf{P} = \left(\frac{n_1 p_1 + n_2 p_2}{n_1 + n_2}\right) \times 100, \ \mathbf{Q} = 100 - \mathbf{P}$$

$$Z = \left(\frac{0.051 - 0.019}{0.014}\right) = 2.29 > Z(1.96)$$
 p < 0.05

Conclusion:

There is a significant difference between R_xA and R_xB with respect to their cure rates.

Comparison of two independent Means

(Student's t-test / unpaired t-test)

A t-test is used when we wish to compare two means

Type of data required

Independent Variable	One nominal variable with two levels	
	E.g., (i) boy/girl students; (ii) non-smoking/heav smoking mothers	у
Dependent Variable	Continuous variable	
	E.g., (i) marks obtained by the students in the annual exam; (ii) Birth weight of children	50

Assumptions

- The samples are random & independent of each other
- The independent variable is categorical & contains only two levels
- The distribution of dependent variable is normal. If the distribution is seriously skewed, the t-test may be invalid.
- The variances are equal in both the groups

Example: A study was conducted to compare the birth weights of children born to 15 non-smoking with those of children born to 14 heavy smoking mothers.

Non-smoking Mothers (n = 15)	Heavy smoking Mothers (n = 14)
3.99	3.18
3.79	2.84
3.60	2.90
3.73	3.27
3.21	3.85
3.60	3.52
4.08	3.23
3.61	2.76
3.83	3.60
3.31	3.75
4.13	3.59
3.26	3.63
3.54	2.38
3.51	2.34
2.71	



Checking the Normality





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. ttest NonSmokers == Heavysmokers, unpaired

Two-sample t test with equal variances

variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]	
NonSmo~s Heavys~s	15 14	3.593333 3.202857	.0957261 .1316774	.3707457 .4926916	3.388021 2.918385	3.798645 3.487329	
combined	29	3.404828	.0872692	.4699591	3.226065	3.58359	
diff		.3904762	.1611908		.0597401	.7212123	
diff = mean(NonSmokers) - mean(Heavysmokers) t = 2.4224 Ho: diff = 0 degrees of freedom = 27							
Ha: d ⁴ Pr(T < t)	iff < 0) = 0.9888	Pr(Ha: diff != T > t) =	0.0224	Ha: d Pr(T > t	iff > 0) = 0.0112	

How to report the results?

	Heavy smoking mothers (n=14)		g Non-smoking mothers (n=15)		Diff in means (95% CI)	P-Value
	Mean	SD	Mean	SD		
Birth weight of children	3.20	0.49	3.60	0.37	0.4 (0.06 – 0.72)	0.022

The difference between birth weight of children born to non-smoking and heavy smoking mothers found by chance is only 2 in a 100 times.

Two groups of paired Observations

Paired t-test

• Same individuals are studied more than once in different circumstances

eg. Measurements made on the same people before and after intervention

- The outcome variable should be continuous
- The difference between pre post measurements should be normally distributed

A study was carried to evaluate the effect of the new diet on weight loss. The study population consist of 12 people have used the diet for 2 months; their weights before and after the diet are given below.

	Weight (Kgs)		
Patient No.	Before Diet	After Diet	
1	75	70	
2	60	54	
3	68	58	
4	98	93	
5	83	78	
6	89	84	
7	65	60	
8	78	77	
9	95	90	
10	80	76	
11	100	94	
12	108	100	

The research question asks whether the diet makes a difference?

Paired t test output

ttest Before == After						
Paired t t	est.					
variable	obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
Before After	12 12	83.25 77.83333	4.346934 4.36382	15.05822 15.11672	73.68246 68.22863	92.81754 87.43804
diff	12	5.416667	.6210735	2.151462	4.049693	6.78364
mean(Ho: mean((diff) = mea (diff) = 0	an(Before – .	After)	degrees	t : of freedom :	8.7215 11
Ha: mean(Pr(T < t)	(diff) < 0) = 1.0000	Ha Pr(: mean(diff) T > t) = (!= 0 0.0000	Ha: mean Pr(T > t)	(diff) > 0) = 0.0000

Clinical Significance Vs Statistical Significance

A possible antipyretic is tested in patients with the common cold.

500 receive the candidate drug

500 receive a placebo control

Temperatures measured 4 hours after dosing

	Ν	Mean	StDev	SE Mean	
Drug	500	39.950	0.653	0.029	
Control	500	40.058	0.699	0.031	
					p value = 0.0 ²

Statistical Significance?	Yes. Probably there is a reduction in temperature
Clinical Significance?	NO. Temperature only fell by about 0.1°c

Because the sample size is so large we are able to detect a very small change in temperature

Review questions:

Q1. Power in statistical terms, represents:

- a) False negative error
- b) Type 1 error
- c) 1-beta
- d) Sample size

- Q2. The basic goal of hypothesis testing is:
- a) To confirm alternate hypothesis
- b) To determine if there is a meaningful difference in outcome between different groups
- c) To establish a p value
- d) To establish alpha value

Q3. Six volunteers have gone on high-oat bran cholesterol lowering die for 3 months. Pretrial and posttrial cholesterol values are as follows:

	Cholesterol levels (mg/dl)		
Subject	Pretrial	Postrial	
1	180	182	
2	225	220	
3	243	241	
4	150	140	
5	212	222	
6	218	216	

In the trial, what is the appropriate test of significance?

- a) Critical ratio
- b) Z test
- c) Paired t test
- d) ANOVA

Q4. Statistical significance is achieved when:

- a) Alpha is greater than or equal to p
- b) Beta is greater than or equal to alpha
- c) p is greater than or equal to alpha
- d) p is greater than or equal to beta

Q5. For a test of CA breast treatment, alpha is set at 0.01 and beta is set at 0.30. in a two tailed test, the new treatment is superior to the standard of care at p=0.04, producing results that are 1 point less effective on a scale in 5% of patients. This result:

- a) shows a statistically significant difference between therapies
- b) Shows a clinically meaningful difference between therapies
- c) Would be less significant if the test were one tailed
- d) Favours continued use of the standard care in clinical practice

• Q6 Which of the following is a type of hypothesis test that is used to compare the means of two independent groups?

a) T-test

b) ANOVA

c) Chi-squared test

d) Paired t-test

- Q7 Alpha, in statistical term means
- a) False Negative error
- b) Sample Size
- c) Type I error
- d) 1-beta

- Q8 Which of the following is a characteristic of a good research hypothesis?
 - a) It is a statement of fact
 - b) It is a general statement
 - c) It is a specific and testable statement
 - d) It is a vague statement

• Q9 Which of the following is a correct interpretation of a p-value of 0.02?

- a) There is a 2% chance that the null hypothesis is true
- b) There is a 98% chance that the alternative hypothesis is true
- c) The observed result is 2 standard deviations away from the mean
- d) The probability of obtaining the observed result or a more extreme result, assuming the null hypothesis is true, is 2%

• Q10 Which of the following is an example of a two-tailed hypothesis test?

a) Testing whether the mean of a sample is greater than a specified value

b) Testing whether the mean of a sample is less than a specified value

c) Testing whether the proportion of successes in a sample is greater than a specified value

d) Testing whether the mean of a sample is different from a specified value

Answers

- Q1:c
- Q2:b
- Q3:c
- Q4:a
- Q5:d
- Q6:a
- Q7:c
- Q8:c
- Q9: d
- Q10:d

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