How many study subjects are required ?
(Estimation of Sample size)
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Objectives of this session: Students able to

- (1) know the importance of sample size in a research project.
- (2) understand the simple mathematics & assumptions involved in the sample size calculations.
- (3) apply sample size methods appropriately in their research projects.

Why to calculate sample size?

- To show that under certain conditions, the hypothesis test has a good chance of showing a desired difference (if it exists)
- To show to that the study has a reasonable chance to obtain a conclusive result
- To show that the necessary resources (human, monetary, time) will be minimized and well utilized

Am I going to reach my study objectives?

- I have 4 months to finish my research project, of which only one week is for data collection
- I think I can get data on 50 subjects in a week
- Is 50 a sufficient number of subjects to test my hypothesis with the significance level I want?

Before We Can Determine Sample Size We Need To Answer The Following:

1. What is the main purpose of the study?
2. What is the primary outcome measure?
   Is it a continuous or dichotomous outcome?
3. How will the data be analyzed to detect a group difference?
4. How small a difference is clinically important to detect?

5. How much variability is in our population?

6. What is the desired α and β?

7. What is the anticipated drop out and non-response % ?

Where do we get this knowledge?

- Previous published studies
- Pilot studies
- If information is lacking, there is no good way to calculate the sample size

- Type I error: Rejecting H_0 when H_0 is true
- α: The type I error rate.
- Type II error: Failing to reject H_0 when H_0 is false
- β: The type II error rate
- Power $(1 - \beta)$: Probability of detecting group difference,given the size of the effect (Δ) and the sample size of the trial (N)

Diagnosis and statistical reasoning

	Disease status Present	Absent
Test result		
+ve	True +ve (sensitivity)	False +ve
-ve	False –ve	True -ve (Specificity)

Test result	**Significance Difference is** Present (Ho *not* true)	Absent (Ho is true)
Reject Ho	No error $1-\beta$	Type I err. α
Accept Ho	Type II err. β	No error $1-\alpha$

α : significance level

$1-\beta$: power

Estimation of Sample Size by Three ways:

By using

(1) Formulae (manual calculations)

(2) Sample size tables or Nomogram

(3) Softwares

Scenario 1
Precision

All studies

Scenario 2
Power

Descriptive

Hypothesis testing

Sample surveys

Simple - 2 groups

Complex studies

SAMPLE SIZE FOR ADEQUATE PRECISION

- In a descriptive study,
- Summary statistics (mean, proportion)
- Reliability (or) precision
- By giving “confidence interval”
- Wider the C.I – sample statistic is not reliable and it may not give an accurate estimate of the true value of the population parameter

Sample size formulae for reporting precision

For single mean : $n = Z^2_\alpha S^2 / d^2$

where S=sd (σ)

For a single proportion : $n = Z^2_\alpha P(1-P)/d^2$

Where , $Z\alpha$ =1.96 for 95% confidence level

$Z\alpha$ = 2.58 for 99% confidence level

Example 1 (Single mean)

A study is to be performed to determine a certain parameter in a community. From a previous study a sd of 46 was obtained.

If a sample error of up to 4 is to be accepted. How many subjects should be included in this study at 99% level of confidence?

Answer

$n = (Z_{\alpha/2})^2\, \sigma^2 / d^2$

σ: standard deviation = 46

d: the accuracy of estimate (how close to the true mean)= given sample error =4

$Z_{\alpha/2}$: A Normal deviate reflects the type I error.
For 99% the critical value =2.58

$$n = \frac{2.58^2 \text{ x } 46^2}{4^2} = 880.3 \sim 881$$

Example 2 (Single proportion)

It was desired to estimate proportion of anemic children in a certain preparatory school. In a similar study at another school a proportion of 30 % was detected.

Compute the minimal sample size required at a confidence limit of 95% and accepting a difference of up to 4% of the true population.

Answer

$n = (Z_{\alpha/2})^2 \, p(1-p) \,/\, d^2$

p: proportion to be estimated = 30% (0.30)

d: the accuracy of estimate (how close to the true proportion) = 4% (0.04)

$Z_{\alpha/2}$: A Normal deviate reflects the type I error

For 95% the critical value =1.96

$$n = \frac{1.96^2 \text{ x } 0.3(1-0.3)}{(0.04)^2} = 504.21 \sim 505$$

Scenario 1
Precision

All studies

Scenario 2
Power

Descriptive

Sample surveys

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Simple - 2 groups

Complex studies

Scenario 2

Three bits of information required to determine the sample size

- Type I & II errors
- Clinical effect
- Variation

Clinical Effect Size

"What is a meaningful difference between the groups"

- It is truly an estimate and often the most challenging aspect of sample size planning
- Large difference – small sample size
- Small differences – large sample size

- Cost/benefit

Variability

Variation

All statistical tests are based on the following ratio:

$$\text{Test Statistic} = \frac{\text{Difference between parameters}}{v / \sqrt{n}}$$

As n ↑ $\quad$ v/√n ↓ $\quad$ Test statistic ↑

Sample size formulae

For two means : $n = 2S^2 (Z_\alpha + Z_\beta)^2 / d^2$

where S=sd

For two proportions :

$$n = \frac{(Z_\alpha + Z_\beta)^2 ((p_1 q_1) + (p_2 q_2))}{(p_1 - p_2)^2}, \text{ where } q_1 = (1 - p_1), q_2 = (1 - p_2)$$

$Z\alpha$ = 1.96 for 95% confidence level

$Z\alpha$ = 2.58 for 99% confidence level ;

Z_β = 0.842 for 80% power

Z_β = 1.282 for 90% power

Example 1: *Does the ingestion of large doses of vitamin A in tablet form prevent breast cancer?*

- Suppose we know from our tumor-registry data that incidence rate of breast cancer over a 1-year period for women aged 45 – 49 is 150 cases per 100,000
- Women randomized to Vitamin A vs. placebo

Example 1 continued

- *Group 1*: Control group given placebo pills. Expected to have same disease rate as registry (**150 cases per 100,000**)

- *Group 2*: Intervention group given vitamin A tablets. Expected to have 20% reduction in risk (**120 cases per 100,000**)

- Want to compare incidence of breast cancer over 1-year

- *Planned statistical analysis*: Chi-square test to compare two proportions from independent samples

$$H_0: p_1 = p_2 \quad \text{vs.} \quad H_A: p_1 \neq p_2$$

Sample Size Formula

To Compare Two Proportions From Independent Samples: H_0: $p_1=p_2$

1. α level
2. β level (1 – power)
3. Expected population proportions (p_1, p_2)

Example 1: *Does ingestion of large doses of vitamin A prevent breast cancer?*

- **Test H_0: $p_1 = p_2$ vs. H_A $p_1 \neq p_2$**
- **Assume 2-sided test with α=0.05 and 80% power**

- **p_1 = 150 per 100,000 = .0015**
- **p_2 = 120 per 100,000 = .0012 (20% rate reduction)**
- **$\Delta = p_1 - p_2$ = .0003**
- **$z_{1-\alpha/2}$ = 1.96 $z_{1-\beta}$ = .84**

- **n per group = 234,882**

- **Too many to recruit in one year!**

Example 2: *Does a special diet help to reduce cholesterol levels?*

- Suppose an investigator wishes to determine sample size to detect a 10 mg/dl difference in cholesterol level in a diet intervention group compared to a control (no diet) group

- Subjects with baseline total cholesterol of at least 300 mg/dl randomized

Example 2 continued

- *Group 1*: A six week diet intervention
- *Group 2*: No changes in diet

- Investigator wants to compare total cholesterol at the end of the six week study

- *Planned statistical analysis*: two sample t-test (for independent samples)(comparison of two means)

$$H_0: \mu_1 = \mu_2 \quad \text{vs.} \quad H_A: \mu_1 \neq \mu_2$$

Sample Size Formula

To Compare Two Means From Independent Samples: H_0: $\mu_1 = \mu_2$

1. α level
2. β level (1 – power)
3. Expected population difference ($\Delta = |\mu_1 - \mu_2|$)
4. Expected population standard deviation (σ_1, σ_2)

Continuous Outcome

(2 Independent Samples)

- Test H_0: $\mu_1 = \mu_2$ vs. H_A: $\mu_1 \neq \mu_2$
- Two-sided alternative and equal allocation
- Assume outcome normally distributed with:

mean μ_1 and variance σ_1^2 in Group 1
mean μ_2 and variance σ_2^2 in Group 2

$$n_{per/group} = \frac{\left(\sigma_1^2 + \sigma_2^2\right)\left(z_{1-\alpha/2} + z_{1-\beta}\right)^2}{\Delta^2}$$

Example 2: ***Does a special diet help to reduce cholesterol levels?***

- Test H_0: $\mu_1=\mu_2$ vs. H_A : $\mu_1\neq\mu_2$
- Assume 2-sided test with α=0.05 and 90% power

- $\Delta = \mu_1 - \mu_2 = 10$ mg/dl
- $\sigma_1 = \sigma_2 = (50$ mg/dl$)$
- $z_{1-\alpha/2} = 1.96 \quad z_{1-\beta} = 1.28$

- n per group = 525

- Suppose 10% loss to follow-up expected, adjust n = 525 / 0.9 = 584 per group

Example 3

- A study is to be done to determine effect of 2 drugs (A and B) on blood glucose level. From previous studies using those drugs, Sd of BGL of 8 and 12 g/dl were obtained respectively.
- A significant level of 95% and a power of 90% is required to detect a mean difference between the two groups of 3 g/dl. How many subjects should be include in each group?

Answer

$$n = \frac{(SD1 + SD2)^2}{\Delta^2} * f(\alpha,\beta)$$

$$n = \frac{(8^2 + 12^2)x10.5}{3^2} = 242.6 \sim 243$$

in each group

The following steps constitute a pragmatic approach to decision taking on Sample size:

(1) Remember that there is no stock answer.

(2) Initiate early discussion among research team members.

(3) Use correct assumptions – consider various possibilities.

(4) Consider other factors also– eg., availability of cases, cost, time.

(5) Make a balanced choice

(6) Ask if this number gives you a reasonable prospect of coming to useful conclusion.

(7) If yes, proceed if no, reformulate your problem for study.

- SAMPLE SIZE:

  How many subjects are needed to assure a given probability of detecting a statistically significant effect of a given magnitude if one truly exists?

- POWER:

  If a limited pool of subjects is available, what is the likelihood of finding a statistically significant effect of a given magnitude if one truly exists?

To conclude,

- Define research question well
- Consider study design, type of response variable, and type of data analysis
- Decide on the type of difference or change you want to detect (make sure it answers your research question)
- Choose α and β
- **Use appropriate equation for sample size calculation or sample size tables/ nomogram or software.**