How many study subjects are required ?
(Estimation of Sample size)

By

Dr.Shaik Shaffi Ahamed
Professor
Dept. of Family & Community Medicine
College of Medicine
King Saud University

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 the funding agency 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

What do I need to know to calculate sample size?

- Most Important: sample size calculation is an educated guess
- It is more appropriate for studies involving hypothesis testing
- There is no magic involved; only statistical and mathematical logic and some algebra
- Researchers need to know something about what they are measuring and how it varies in the population of interest

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

- 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?

- 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)

	Significance Difference is	
	Present (Ho *not* true)	Absent (Ho is true)
Test result		
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

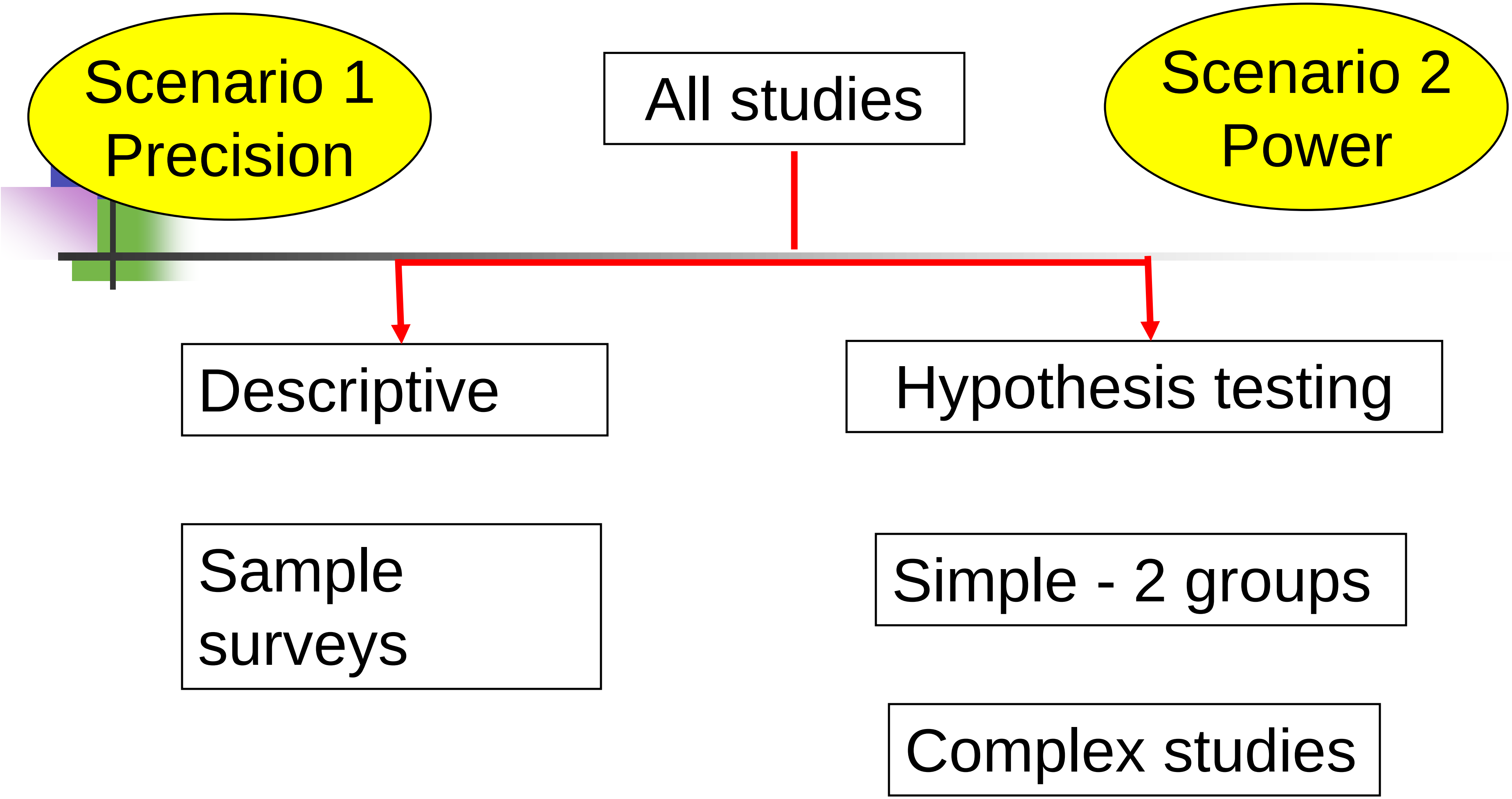
Estimation of Sample Size by Three ways:

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

Problem 1 (Single mean)

A study is to be performed to determine a certain parameter(BMI) 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

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

Problem 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

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

- **WHAT LEVEL OF PRECISION CAN BE ACHIEVED WITH A GIVEN SAMPLE SIZE ?**

- **FIXED BUDGETS**
- **LIMITED RESOURCES AND TIME**

- $d = Z_{\alpha/2} * (\sigma/\sqrt{n})$ for a mean
- $d = Z_{\alpha/2} * \sqrt{p(1-p)/n}$ for a proportion

- Example: For the estimation of the proportion of Anemic children in a preparatory school, what width 95% C.I. would be achieved with a sample of 200 children ?

Solution:

Here $p = 0.30$, $n = 200$ and $Z\alpha/2 = 1.96$

$$d = 1.96 * \sqrt{0.3\,(1-0.3)/200} = 0.064$$

So we would be within ± 6% of the true proportion.

Scenario 2

Three bits of information required to determine the sample size

Type I & II errors

Clinical effect

Variation

Quantities related to the research question (defined by the researcher)

- α = Probability of rejecting H_0 when H_0 is true
- α is called significance level of the test
- β = Probability of not rejecting H_0 when H_0 is false
- $1-\beta$ is called statistical power of the test

- Researcher fixes probabilities of type I and II errors
  - Prob (type I error) = Prob (reject H_0 when H_0 is true) = α
    - Smaller error $\Rightarrow$ greater precision $\Rightarrow$ need more information $\Rightarrow$ need larger sample size
  - Prob (type II error) = Prob (don't reject H_0 when H_0 is false) = β
  - Power =1- β
    - More power $\Rightarrow$ smaller error $\Rightarrow$ need larger sample size

Quantities related to the research question (defined by the researcher)

- Size of the measure of interest to be detected
  - Difference between two or more means
  - Odds ratio
  - Change in R^2, etc

- The magnitude of these values depend on the research question and objective of the study (for example, clinical relevance)

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 ↑

Example 1: *Does the consumption 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)

Dichotomous Outcome (2 Independent Samples)

- Test H_0: $p_1 = p_2$ vs. H_A: $p_1 \neq p_2$
- Assuming two-sided alternative and equal allocation

$$n_{per/group} = \left[\frac{z_{1-\alpha/2}\sqrt{2\bar{p}\bar{q}} + z_{1-\beta}\sqrt{p_1q_1 + p_2q_2}}{\Delta} \right]^2$$

- p_1, p_2 = projected true probabilities of "success" in the two groups
- $q_1 = 1 - p_1,\ q_2 = 1 - p_2$
- $\Delta = p_1 - p_2$
- $\bar{p} = (p_1 + p_2)/2,\ \bar{q} = 1 - \bar{p}$
- $z_{1-\alpha/2}$ is the N(0,1) cutoff corresponding to α
- $z_{1-\beta}$ is the N(0,1) cutoff corresponding to β

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 $\alpha = 0.05$ and 90% power
- $\Delta = \mu_1 - \mu_2 = 10$ mg/dl
- $\sigma_1 = \sigma_2 = (50$ mg/dl$)$
- $z_{1-\alpha/2} = 1.96$ $z_{1-\beta} = 1.28$
- n per group = 525
- Suppose 10% loss to follow-up expected, adjust n = 525 / 0.9 = 584 per group

Problem (comparison of two means)

- 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

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

$$n = \frac{(8^2 + 12^2) x 10.5}{3^2} = 242.6 \sim 243$$

in each group

Comparison of two means

- Objective:

To observe whether feeding milk to 5 year old children enhances growth.

Groups:

Extra milk diet

Normal milk diet

Outcome:

Height (in cms.)

Assumptions or specifications:

Type-I error (α) =0.05

Type-II error (β) = 0.20

i.e., Power($1-\beta$) = 0.80

Clinically significant difference (Δ) =0.5 cm.,

Measure of variation (SD.,) =2.0 cm.,

(from literature or “Guesstimate”)

Using the appropriate formula:

$$N = \frac{2(SD)^2}{\Delta^2} f(\alpha,\beta)$$

$$= \frac{2(2)^2}{(0.5)^2} \; 7.9$$

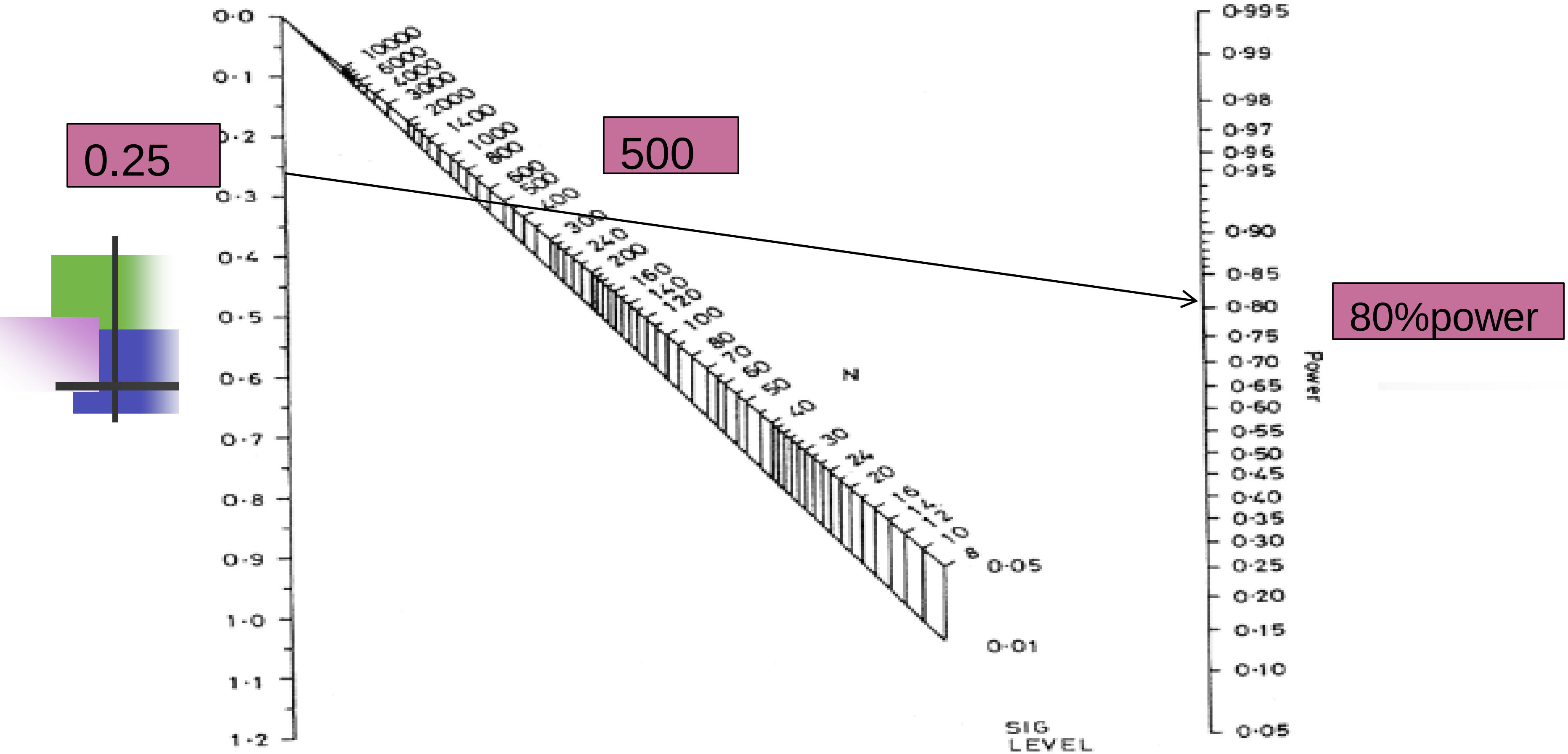
$= 252.8$ (in each group)

Simple Method:
--- Nomogram

$$\text{Standardized difference} = \frac{\text{Target difference}}{\text{Standard deviation}}$$

$$= 0.5/2.0 \quad = 0.25$$

Figure 1



Nomogram for calculating sample size or power. Reproduced from Altman [5], with permission.

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.

Summary

- 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.

Any Q's