Teaching Mathematics and Statistics in Sciences, IPA HU-SRB/0901/221/088 - 2011

Biostatistics

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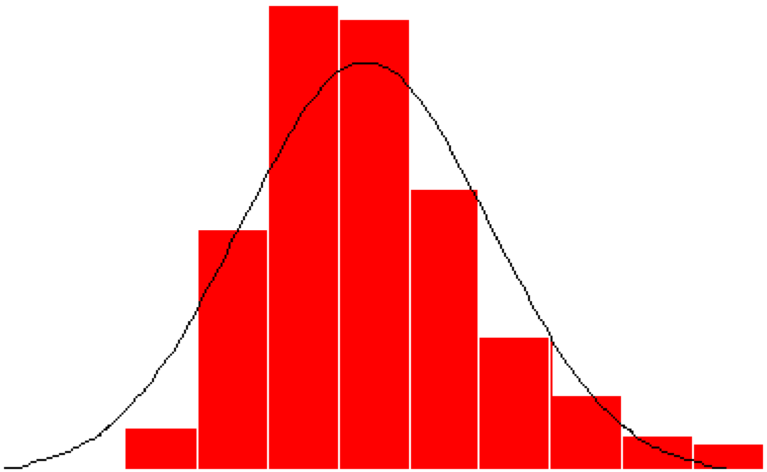
Hypothesis tests II.
Two sample t-test, statistical errors.



Motivating example

- Two lecturers argue about the mean age of the first year medical students. Is the mean age for boys and girls the same or not?
  - Lecturer#1 claims that the mean age boys and girls is the same.
  - Lecturer#2 does not agree.
  - Who is right?
- Statistically speaking: there are two populations:
  - the set of ALL first year boy medical students (anywhere, any time)
  - the set of ALL first year girl medical students (anywhere, any time)
- Lecturer#1 claims that the population means are equal: $\mu_{boys} = \mu_{girls}$.
- Lecturer#2 claims that the population means are not equal: $\mu_{boyys} \neq \mu_{girls}$.

Independent samples

- compare males and females
- compare two populations receiving different treatments
- compare healthy and ill patients
- compare young and old patients
- ……

Experimental design of *t*-tests

- **Paired t-test**
- Each subject are measured twice

1st	2nd
x_1	y_1
x_2	y_2
…	…
x_n	y_n

- **Two-sample t-test**
- Each subject is measured once, and belongs to one group .

Group	Measurement
1	x_1
1	x_2
…	…
1	x_n
2	y_1
2	y_2
…	…
2	y_m

Sample size is not necessarily equal

Student's t-tests

- **General purpose**. Student's t-tests examine the mean of normal populations. To test hypotheses about the population mean, they use a test-statistic ***t*** that follows Student's t distribution with a given degrees of freedom if the nullhypothesis is true.
- **One-sample t-test.** There is one sample supposed to be drawn from a normal distribtuion. We test whether the mean of a normal population is a given constant:
  - H0: μ=c
- **Paired t-test (=one-sample t-test for paired differences).** There is only one sample that has been tested twice (before and after the treatment) or when there are two samples that have been matched or "paired".We test whether the mean difference between paired observations is zero:
  - H0: $\mu_{differerence}$=0
- **Two sample t-test (or independent samples t-test).** There are two independent samples, coming from two normal populations. We test whether the two population means are equal:
  - H0: μ_1= μ_2

Testing the mean of two independent samples from normal populations: two-sample *t*-test

- Independent samples:
  - Control group, treatment group
  - Male, female
  - Ill, healthy
  - Young, old
  - etc.
- Assumptions:
  - Independent samples : $x_1, x_2, \ldots, x_n$ and $y_1, y_2, \ldots, y_m$
  - the x_i-s are distributed as $N(\mu_1,\sigma_1)$ and the y_i-s are distributed as $N(\mu_2,\sigma_2)$.
- H_0: $\mu_1=\mu_2$, H_a: $\mu_1\neq\mu_2$

Decision rules

- Confidence intervals: there are confidence intervals for the difference (we do not study)
- Critical points
- P-values

If $p<0.05$, we say that the result is statistically significant at 5% level:
i.e. the effect would occur by chance less than 5% of the time

Evaluation of two sample t-test depends on equality of variances

The case when the population standard deviations are equal

- Assumptions:
  - 1. Both populations are normal.
  - 2. The variances of the two populations are equal ($\sigma_1 = \sigma_1 = \sigma$).
    - That is the x_i-s are distributed as $N(\mu_1, \sigma)$ and the y_i-s are distributed as $N(\mu_2, \sigma)$
- H_0: $\mu_1 = \mu_2$, H_a: $\mu_1 \neq \mu_2$
- If H_0 is true, then

$$t = \frac{\overline{x} - \overline{y}}{s_p \sqrt{\frac{1}{n} + \frac{1}{m}}} = \frac{\overline{x} - \overline{y}}{s_p} \cdot \sqrt{\frac{nm}{n+m}} . \qquad s_p^2 = \frac{(n-1) \cdot s_x^2 + (m-1) \cdot s_y^2}{n+m-2}$$

has Student's t distribution with **n+m-2** degrees of freedom.

- Decision:
- If $|t| > t_{\alpha, n+m-2}$, the difference is significant at α level, we reject H0
- If $|t| < t_{\alpha, n+m-2}$, the difference is not significant at α level, we do not reject H0

The case when the standard deviations are not equal

- Both populations are approximately normal.
- 2. The variances of the two populations are not equal ($\sigma_1 \neq \sigma_1$).
- That is the *xi*-s are distributed as $N(\mu_1, \sigma_1)$ and the *yi*-s are distributed as $N(\mu_2, \sigma_2)$

- H_0: $\mu_1 = \mu_2$, H_a: $\mu_1 \neq \mu_2$
- If H_0 is true, then

$$d = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{s_x^2}{n} + \frac{s_y^2}{m}}}$$

$$df = \frac{(n-1)\cdot(m-1)}{g^2\cdot(m-1) + (1-g^2)\cdot(n-1)}$$

$$g = \frac{\frac{s_x^2}{n}}{\frac{s_x^2}{n} + \frac{s_y^2}{m}}$$

has Student t distribution with ***df*** degrees of freedom.

- Decision:
- If $|t| > t_{\alpha, n+m-2}$, the difference is significant at α level, we reject H0
- If $|t| < t_{\alpha, n+m-2}$, the difference is not significant at α level, we do not reject H0

Comparison of the variances of two normal populations: F-test

- H_0: $\sigma_1 = \sigma_2$
- H_a: $\sigma_1 > \sigma_2$ (one sided test)
- F: the higher variance divided by the smaller variance:

$$F = \frac{\max(s_x^2, s_y^2)}{\min(s_x^2, s_y^2)}$$

- Degrees of freedom:
  - 1. Sample size of the nominator-1
  - 2. Sample size of the denominator-1
- Decision based on F-table
  - If $F > F_{\alpha,table}$, the two variances are significantly different at α level

Table of the F-distribution α=0.05

Nominator->

Denominator

|

számláló-> nevező↓	1	2	3	4	5	6	7	8	9	10
1	161.4476	199.5	215.7073	224.5832	230.1619	233.986	236.7684	238.8827	240.5433	241.8817
2	18.51282	19	19.16429	19.24679	19.29641	19.32953	19.35322	19.37099	19.38483	19.3959
3	10.12796	9.552094	9.276628	9.117182	9.013455	8.940645	8.886743	8.845238	8.8123	8.785525
4	7.708647	6.944272	6.591382	6.388233	6.256057	6.163132	6.094211	6.041044	5.998779	5.964371
5	6.607891	5.786135	5.409451	5.192168	5.050329	4.950288	4.875872	4.81832	4.772466	4.735063
6	5.987378	5.143253	4.757063	4.533677	4.387374	4.283866	4.206658	4.146804	4.099016	4.059963
7	5.591448	4.737414	4.346831	4.120312	3.971523	3.865969	3.787044	3.725725	3.676675	3.636523
8	5.317655	4.45897	4.066181	3.837853	3.687499	3.58058	3.500464	3.438101	3.38813	3.347163
9	5.117355	4.256495	3.862548	3.633089	3.481659	3.373754	3.292746	3.229583	3.178893	3.13728
10	4.964603	4.102821	3.708265	3.47805	3.325835	3.217175	3.135465	3.071658	3.020383	2.978237

Example

Control group	Treated group
170	120
160	130
150	120
150	130
180	110
170	130
160	140
160	150
	130
	120
n=8	n=10
$\bar{x}=162.5$	$\bar{y}=128$
$s_x=10.351$	$s_y=11.35$
$s_x^2=107.14$	$s_y^2=128.88$

$$F=\frac{128.88}{107.14}=1.2029,$$

Degrees of freedom 10-1=9, 8-1=7, critical value int he F-table is $F_{\alpha,9,7}=3.68$. As 1.2029<3.68, the two variances are considered to be equal, the difference is not significanr.

$$s_p^2=\frac{7\cdot 107.14+9\cdot 128.88}{10+8-2}=\frac{749.98+1160}{16}=119.37$$

$$t=\frac{162.5-128}{\sqrt{119.37}}\cdot\sqrt{\frac{10\cdot 8}{18}}=\frac{34.5}{10.92}\cdot\sqrt{4.444}=6.6569$$

Our computed test statistic t = 6.6569 , the critical value int he table $t_{0.025,16}=2.12$. As 6.6569>2.12, we reject the null hypothesis and we say that the difference of the two treatment means is significant at 5% level

Result of SPSS

Group Statistics

	csoport	N	Mean	Std. Deviation	Std. Error Mean
BP	Kontroll	8	162.5000	10.35098	3.65963
	Kezelt	10	128.0000	11.35292	3.59011

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
BP	Equal variances assumed	.008	.930	6.657	16	.000	34.50000	5.18260	23.51337	45.48663
	Equal variances not assumed			6.730	15.669	.000	34.50000	5.12657	23.61347	45.38653

Two sample t-test, example 2.

- A study was conducted to determine weight loss, body composition, etc. in obese women before and after 12 weeks in two groups:
- Group I. treatment with a very-low-calorie diet .
- Group II. no diet
- Volunteers were randomly assigned to one of these groups.
- We wish to know if these data provide sufficient evidence to allow us to conclude that the treatment is effective in causing weight reduction in obese women compared to no treatment.

Two sample t-test, cont.
Data

Group	Patient	Change in body weight
Diet	1	-1
	2	5
	3	3
	4	10
	5	6
	6	4
	7	0
	8	1
	9	6
	10	6
Mean		4.
SD		3.333
No diet	11	2
	12	0
	13	1
	14	0
	15	3
	16	1
	17	5
	18	0
	19	-2
	20	-2
	21	3
Mean		1
SD		2.145

Two sample t-test, example, cont.

- HO: $\mu_{diet}=\mu_{control,}$ (the mean change in body weights are the same in populations)
- H_a: $\mu_{diet} \neq \mu_{control}$ (the mean change in body weights are different in the populations)
- Assumptions:
  - normality (now it cannot be checked because of small sample size)
  - Equality of variances (check: visually compare the two standard deviations)

Two sample t-test, example, cont.

- Assuming equal variances, compute the t test- statistic: t=2.477

$$t = \frac{\bar{x} - \bar{y}}{s_p \sqrt{\frac{1}{n} + \frac{1}{m}}} = \frac{\bar{x} - \bar{y}}{s_p} \cdot \sqrt{\frac{nm}{n+m}} = \frac{4-1}{\sqrt{\frac{9 \cdot 3.3333^2 + 10 \cdot 2.145^2}{9+10}}} \sqrt{\frac{10 \cdot 11}{10+11}} = \frac{3}{\sqrt{\frac{99.999 + 46.01025}{19}}} \sqrt{5.238} = 2.477$$

- Degrees of freedom: 10+11-2=19
- Critical t-value: $t_{0.05,19}$=2.093
- Comparison and decision:
  - |t|=2.477>2.093(=$t_{0.05,19}$), the difference is significant at 5% level
- p=0.023<0.05 the difference is significant at 5% level

SPSS results

Group Statistics

	group	N	Mean	Std. Deviation	Std. Error Mean
Change in body mass	Diet	10	4.0000	3.33333	1.05409
	Control	11	1.0000	2.14476	.64667

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Change in body mass	Equal variances assumed	1.888	.185	2.477	19	.023	3.00000	1.21119	.46495	5.53505
	Equal variances not assumed			2.426	15.122	.028	3.00000	1.23665	.36600	5.63400

Comparison of variances.
p=0.185>0.05, not significant.
We accept the equality of variances

Comparison of means (t-test).
1st row: equal variances assumed.
t=2.477, df=19, p=0.023
The difference in mean weight loss is significant at 5% level

Comparison of means (t-test). 2nd row: equal variances not assumed.
As the equality of variances was accepted, we do not use the results from this row.

Example from the medical literature

Differential Effect of Urotensin II on Vascular Tone in Normal Subjects and Patients With Chronic Heart Failure

Melissa Lim, BBiomedSc; Suzy Honisett, BSc, PhD; Christopher D. Sparkes, BBiomedSc;
Paul Komesaroff, MBBS, PhD, FRACP;
Andrew Kompa, BSc, PhD; Henry Krum, MBBS, PhD, FRACP

Statistical Analysis

Data are expressed as mean±SEM. U-II responses within groups were analyzed by trend test. U-II responses between groups were analyzed overall by 2-way, repeated-measures ANOVA. Comparison between groups for individual doses of iontophoresed agents was by Student's unpaired *t* test. Nonparametric data, for example, gender, were analyzed by χ^2 analysis or Mann-Whitney test, as appropriate. A 2-tailed probability value of <0.05 was considered statistically significant.

Demographic Indexes

Parameter	Normal Subjects (n=14)	Patients With CHF (n=13)	*P*
Age, y	50±4	56±4	0.28
Gender, male/female	5/9	11/2	0.03*
Body mass index, kg/m^2	24.3±0.8	27.3±2.0	0.18
Systolic blood pressure, mm Hg	125±4.0	115±4.0	0.08
Diastolic blood pressure, mm Hg	77±2.0	70±4.0	0.14
Heart rate, bpm	68±3.0	71±3.0	0.50
Sodium, mmol/L	143±0.55	141±0.83	0.05*
Potassium, mmol/L	4.31±0.06	4.52±0.09	0.06
Chloride, mmol/L	103±0.71	101±1.0	0.25
Bicarbonate, mmol/L	26.8±0.42	26.6±0.65	0.77
Urea, mmol/L	6.28±0.56	7.08±0.36	0.25
Creatinine, mmol/L	0.07±0.005	0.08±0.006	0.22
NT-proBNP, pg/mL	57.0±28.8	1162±591	0.004*

*$P<0.05$ for differences in gender, serum sodium, and N-terminal pro–brain natriuretic peptide (NT-proBNP) between groups.

Lim et al U-II in Heart Failure *1213*

Demographic Indexes

Parameter	Normal Subjects (n=14)	Patients With CHF (n=13)	*P*
Age, y	50±4	56±4	0.28
Gender, male/female	5/9	11/2	0.03*
Body mass index, kg/m²	24.3±0.8	27.3±2.0	0.18
Systolic blood pressure, mm Hg	125±4.0	115±4.0	0.08
Diastolic blood pressure, mm Hg	77±2.0	70±4.0	0.14
Heart rate, bpm	68±3.0	71±3.0	0.50
Sodium, mmol/L	143±0.55	141±0.83	0.05*
Potassium, mmol/L	4.31±0.06	4.52±0.09	0.06
Chloride, mmol/L	103±0.71	101±1.0	0.25
Bicarbonate, mmol/L	26.8±0.42	26.6±0.65	0.77
Urea, mmol/L	6.28±0.56	7.08±0.36	0.25
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NT-proBNP, pg/mL	57.0±28.8	1162±591	0.004*

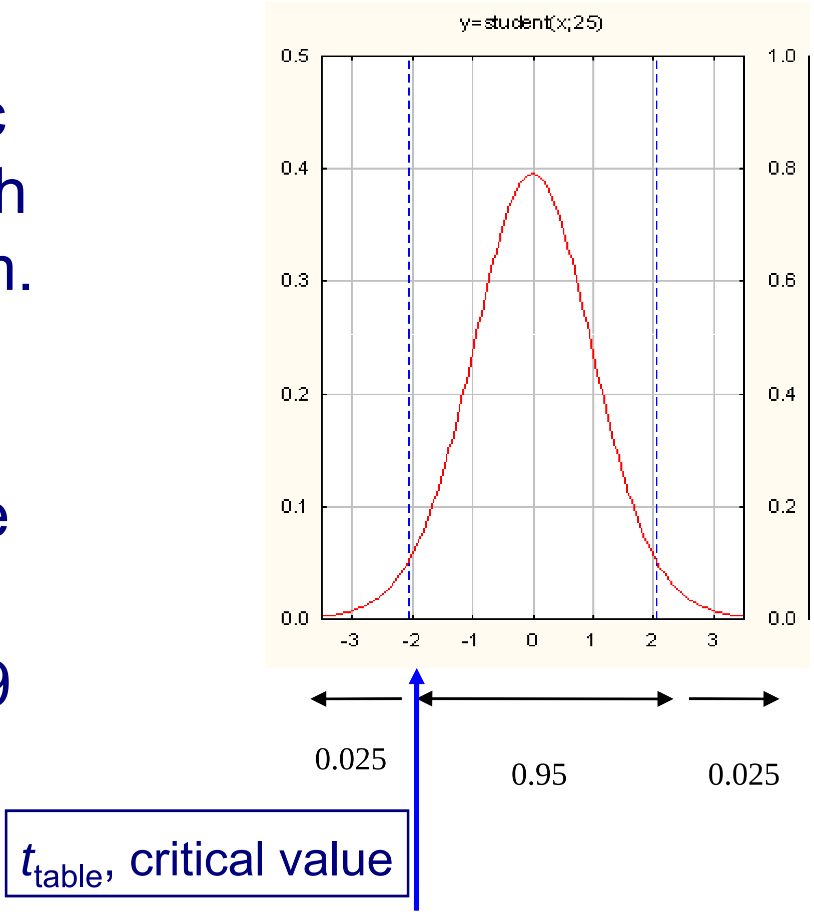
*$P<0.05$ for differences in gender, serum sodium and N-terminal pro–brain natriuretic peptide (NT-proBNP) between groups.

Compare the mean age in the two groups!
The sample means are „similar”. Is this small difference really caused by chance?

- Step 1.
  - H_0: the means in the two populations are equal: $\mu_1=\mu_2$
  - H_A: the means in the two populations are not equal: $\mu_1 \neq \mu_2$
- Step 2.
  - Let $\alpha=0.05$
- Step 3.
  - Decision rule: two-sample t-test.
- Step 4. Decision.
  - Decision based on test statistic:
    - Compute the test statistics: t=-1.059, the degrees of freedom is 14+13-2=25
    - t_{table}=2.059
    - $|t|=1.059<2.059$, the difference is not significant at 5% level.
  - $p=0.28$, $p>0.05$, the difference is not significant at 5% level.

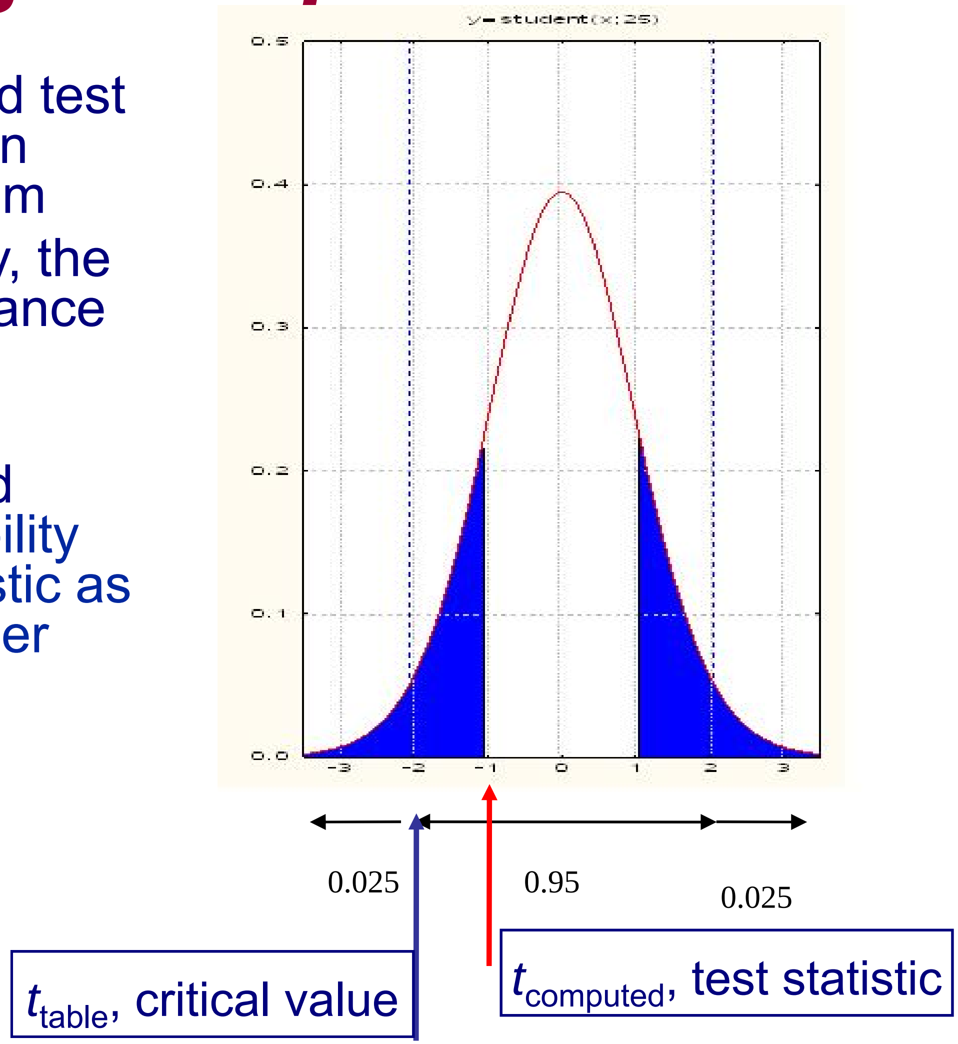
How to get the p-value?

- If H0 is true, the computed test statistic has a t-distribution with 25 degrees of freedom.
- Then with 95% probability, the *t*-value lies in the „acceptance region”
- Check it: now t=-1.059



How to get the *p*-value?

- If H0 is true, the computed test statistic has a t-distribution with 25 degrees of freedom
- Then with 95% probability, the t-value lies in the „acceptance region"
- Check it: now t=-1.059
- The p-value is the shaded area, p=0.28. The probability of the observed test statistic as is or more extreme in either direction when the null hypothesis is true.



How to get the t-value using statistical software – given sample size, sample mean and sample SD?

- Using SPSS, t-test is performed on sample data. Given only sample characteristics, it is difficult to get t.value.
- Excel:

	Group I	Group II
N	14	13
Mean	50	56
SD	4	4
Results		
Mean difference		-6
SE of mean difference		1.540658
Df		25
t-value		-3.89444
two-sided p		0.000649

	Group I	Group II
N	14	13
Mean	50	56
SE	4	4
SD	14.96663	14.42221
Results		
Mean difference		-6
SE of mean difference		5.66493
Df		25
t-value		-1.05915
two-sided p		0.299659

Answer to the motivated example (mean age of boys and girls)

Group Statistics

	Sex	N	Mean	Std. Deviation	Std. Error Mean
Age in years	Male	84	21.18	3.025	.330
	Female	53	20.38	3.108	.427

- The mean age of boys is a litlle bit higher than the mean age of girls. The standard deviations are similar.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Age in years	Equal variances assumed	.109	.741	1.505	135	.135	.807	.536	-.253	1.868
	Equal variances not assumed			1.496	108.444	.138	.807	.540	-.262	1.877

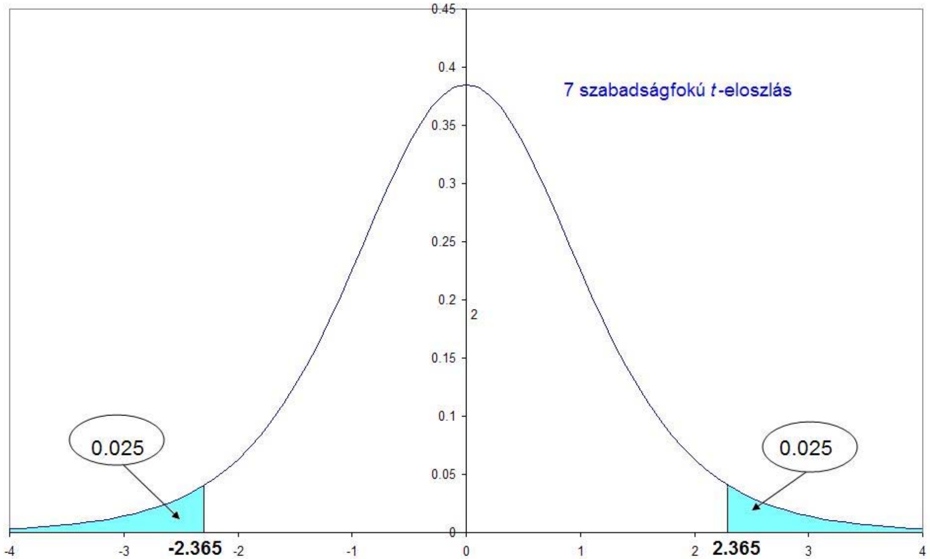
- Comparison of variances (F test for the equality of variances): p=0.741>0.05, not significant, we accept the equality of variances.
- Comparison of means: according to the formula for equal variances, t=1.505. df=135, p=0.135. So p>0.05, the difference is not significant. Althogh the experiencedd difference between the mean age of boys and girls is 0.816 years, this is statistically not significant at 5% level. We cannot show that the mean age of boay and girls are different.

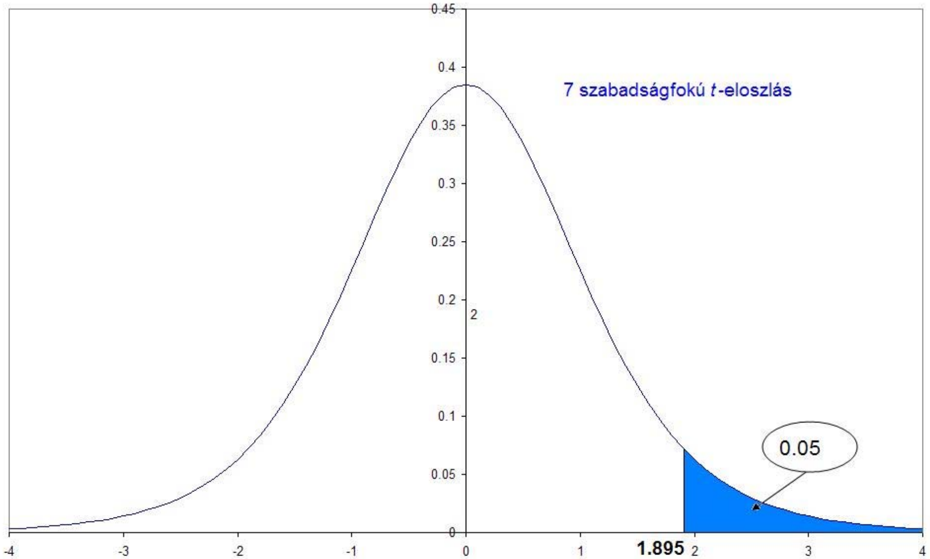
Other aspects of statistical tests

One- and two tailed (sided) tests

- Two tailed test
- H_0: there is no change $\mu_1=\mu_2$,
- H_a: There is change (in either direction) $\mu_1 \neq \mu_2$

- One-tailed test
- H_0: the change is negative or zero $\mu_1 \leq \mu_2$
- H_a: the change is positive (in one direction) $\mu_1 > \mu_2$





Critical values are different. ***p*-values:** *p*(one-tailed)=*p*(two-tailed)/2

Significance

- Significant difference – if we claim that there is a difference (effect), the probability of mistake is small (maximum α- Type I error).
- Not significant difference – we say that there is not enough information to show difference. Perhaps
  - there is no difference
  - There is a difference but the sample size is small
  - The dispersion is big
  - The method was wrong
- Even is case of a statistically significant difference one has to think about its biological meaning

Statistical errors

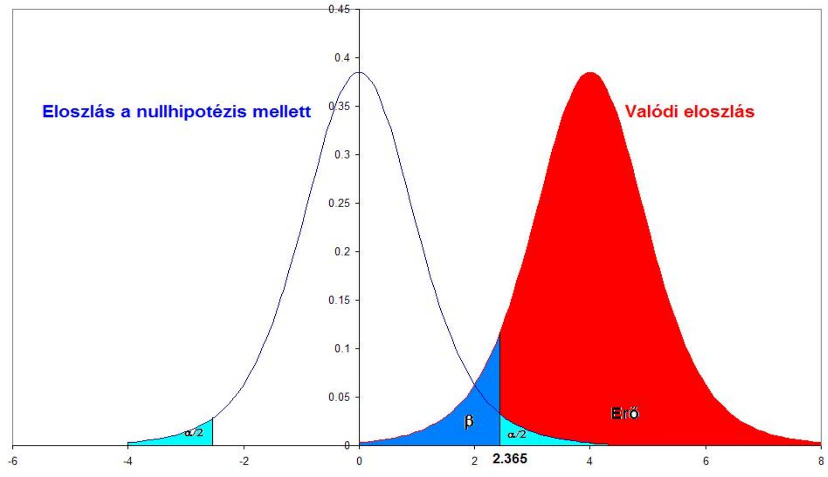
Truth	Decision	
	do not reject H_0	reject H_0 (significance)
H_0 is true	correct	Type I. error its probability: α
H_a is true	Type II. error its probability: β	correct

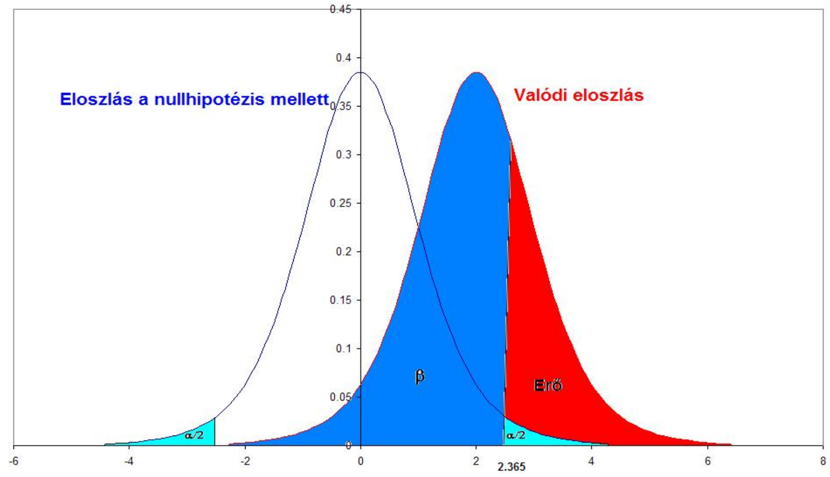
Error probabilities

- The probability of type I error is known (α).
- The probability of type II error is not known (β)
- It depends on
  - The significance level (α),
  - Sample size,
  - The standard deviation(s)
  - The true difference between populations
  - others (type of the test, assumptions, design, ..)
- The power of a test: 1- β

  It is the ability to detect a real effect

The power of a test in case of fixed sample size and α, with two alternative hypotheses





Review questions and problems

- The null- and alternative hypothesis of the two-sample t-test
- The assumption of the two-sample t-test
- Comparison of variances
- F-test
- Testing significance based on t-statistic
- Testing significance based on p-value
- Meaning of the p-value
- One-and two tailed tests
- Type I error and its probability
- Type II error and its probability
- The power of a test
- In a study, the effect of Calcium was examined to the blood pressure. The decrease of the blood pressure was compared in two groups. Interpret the SPSS results

Group Statistics

	treat	N	Mean	Std. Deviation	Std. Error Mean
decr	Calcium	10	5.0000	8.74325	2.76486
	Placebo	11	-.2727	5.90069	1.77913

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
decr	Equal variances assumed	4.351	.051	1.634	19	.119	5.27273	3.22667	-1.48077	12.02622
	Equal variances not assumed			1.604	15.591	.129	5.27273	3.28782	-1.71204	12.25749