Seminars in Applied Statistics for Radiation Cytogenetics and Biodosimetry

Volodymyr Vinnikov

S.P. Grigoriev Institute for Medical Radiology and Oncology of the National Academy of Medical Science of Ukraine

Seminar III. Comparison of datasets

Contents: Parametric and non-parametric methods. Student’s t-test – independent groups or repeated studies. Fisher's test of equality of variances. Non-parametric methods: Mann – Whitney *U* test and Wilcoxon signed-rank *T* test. Chi-squared test, relative risk and odds ratio. ~~Assessment of the effect of acting factor: Detection versus measurement. ANOVA and Kruskal–Wallis *H* test.~~

Parametric methods – based on the use, or analysis, or comparison of certain parameters. Parameters can be mean, dispersion, standard error…
Examples of parametric methods: Student's *t*-test, Fisher's *F*-test of equality of variances, One-way analysis of variance (*ANOVA)*…

Non-parametric methods – don't require parameters. Often are based on the position (rank) of the value in the group. Also suitable for analysis of qualitative (categorial) data.
Examples: Pearson's chi-squared (χ^2) test, Mann – Whitney *U* test (also called the Mann–Whitney–Wilcoxon), Wilcoxon signed-rank *T* test, Kruskal–Wallis *H* test, Kendall rank correlation coefficient τ, etc.

Usually, non-parametric tests are considered having lower statistical power compared to their parametric analogues. The advantages of non-parametric tests are their applicability to small groups and heterogeneous data.

Choosing methods for data comparison between groups

Conditions & Tasks	Parametric methods	Non-parametric methods
Characteristics of group data	$n > 20$; modal classes are clearly present (bell-shaped distribution, even asymmetrical); data are numeric quantities (discrete counts, or continuous values, or frequencies).	$n \leq 20$; no modal classes (flat distribution); data can be of any type, including discrete counts or qualitative (categorial – ordinal or nominal).
Comparison of two independent groups	Student's *t*-test	Mann – Whitney *U* test
Comparison of paired or matched data	Student's paired *t*-test	Wilcoxon signed-rank *T* test
To test for differences between distributions	Fisher's *F*-test of equality of variances	Pearson's χ^2 test
To test for differences among at least three groups	One-way analysis of variance (*ANOVA*)	Kruskal–Wallis *H* test

Student's t-test for the difference of the means in two independent groups, e.g. "Exposed" versus Controls.

t-test: difference between group means (*d*), divided by the error of this difference (s_d)

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s_d} = \frac{d}{s_d}$$

$$s_d = \sqrt{\frac{(n_1 - 1)SE_1^2 + (n_2 - 1)SE_2^2}{n_1 + n_2 - 2}\left(\frac{n_1 + n_2}{n_1 n_2}\right)}$$

The formula of the error of the difference is for groups, which are not equal in size.
SE is standard error of the mean.
Degrees of freedom: $k = n_1 + n_2 - 2$, where *n* is the number of individuals in the group.
Difference is significant if $t > t_{st}$ for given *k*.
The value of t_{st} is taken from the tables.

Students t-test should be used, if the distributions of individual values in the compared group are close to one of parametric distributions, e.g., normal, or binomial, or Poisson.

Data for the "Exposed" group: virtual group from the Seminar II

26 individuals; 9♀ & 17♂. Age ranged 22 – 75 y.; ≈13 000 cells scored; 45 Dic+CR found; individual aberration yields distributed closely to Poisson statistics; weighted group mean yield of aberrations (Dic+CR ± SE) = 0.00347±0.00093 per cell; dispersion σ^2 = 0.0000220.

Data for the "Control" group: GIMRO RCL's historical control

50 individuals; 31♀ & 19 ♂. Age ranged 16 – 58 y.; ≈20 000 cells scored; 16 Dic+CR found; individual aberration yields distributed closely to Poisson statistics; weighted group mean yield of aberrations (Dic+CR ± SE) = 0.00083±0.00015 per cell; dispersion σ^2 = 0.0000011.

In this example:

$d = 0.00347 - 0.00083 = 0.00264$

$s_d = \sqrt{[((25 \times 0.00093^2 + 49 \times 0.00015^2) \times (26 + 50)) / ((26 + 50 - 2) \times 26 \times 50)]} = 0.000134$

$t = 0.00264 / 0.000134 = 19.70$

$k = 26 + 50 - 2 = 74$

For k = *74*	t_{st} = 1.99	2.66	3.40
	$p<0.05$	$p<0.01$	$p<0.001$

($p=1.85 \times 10^{-31}$ by the function T.DIST.RT in MS Excel)

Student's t-test for independent groups: Qualitative data, comparison of frequencies

The groups are not equal in size.

Frequency $f = m / n$, where
m – number of cases in the certain class withing a group containing n persons.

$$t = \frac{d_f}{s_{d_f}} = \frac{f_1 - f_2}{s_{d_f}} = \frac{m_1/n_1 - m_2/n_2}{s_{d_f}}$$

$$s_{d_p} = \sqrt{\frac{m_1 + m_2}{n_1 n_2}\left(1 - \frac{m_1 + m_2}{n_1 + n_2}\right)}$$

Degrees of freedom:
$k = n_1 + n_2 - 2$
Difference is significant if $t > t_{st}$ for given k. The value of t_{st} should be taken from the tables.

Proportion of males in the exposed and control groups:
$f_1 = 17 / 26 = 0.6538$
$f_2 = 19 / 50 = 0.3800$

In our example: $d = 0.2738$; $s_d = 0.1207$;
$t = 2.27$
For $k = 74$ $t_{st} = 1.99 – 2.66 – 3.40$;
$p<0.05$ $p<0.01$ $p<0.001$
($p=0.0131$ by the function T.DIST.RT in MS Excel)

Fisher's test of equality of variances : "Exposed" versus Controls.

The groups are not equal in size.

$$F = \sigma_1^2 / \sigma_2^2 \quad \text{at} \quad \sigma_1^2 \geqslant \sigma_2^2$$

Fisher's test is the ratio of dispersions.
Always F ≥ 1.
Degrees of freedom:
$k_1 = n_1 - 1$; $k_2 = n_2 - 1$.
Difference is significant if $F > F_{st}$ for given k_1 and k_2.
The value of F_{st} is taken from the tables.

In our example:
$F = \sigma^2_1 / \sigma^2_2 = 0.0000220 / 0.0000011 = 20$
$k_1 = n_1 - 1 = 25$
$k_2 = n_2 - 1 = 49$
$F_{st} \approx 1.74$ for $p<0.05$; $F_{st} \approx 2.21$ for $p<0.01$
$F > F_{st}$; $p<0.01$
($p=6.13\times10^{-18}$ by the function F.DIST.RT in MS Excel)

Student's t-test for paired measurements: "before – after" study design.

Example with different numbers of cells scored per person in both studies.

***t*-test: mean difference between the weighted individual yields in the second and the first studies (d_i), divided by the error of this difference (s_d).**
Individual statistical weight is the number of cells scored for person (N_i), divided by the mean number of cells scored per person in the group (N_{mean}) in this study.

$$t = \frac{\bar{d}}{s_d} = \frac{\frac{\sum d_i}{n}}{s_d}$$

$$d_i = \sum (x_{i2} f_{i2} - x_{i1} f_{i1})$$

$$s_d = \sqrt{\frac{\sum (d_i - \bar{d})^2}{n(n-1)}}$$

Degrees of freedom: $k = n - 1$, where n is the number of pairs.
Difference is significant if $t > t_{st}$ for given *k*.
The value of t_{st} is taken from the tables.

Study 1 – Virtual group from the Seminar II

Patient's code	Individial	Cells scored	Aberrations found	Yield	Difference with mean	Square difference	Weight, f_i	Square difference x Weight
1	AAA	1000	0	0.0000	-0.0035	0.00001225	2.00462606	2.45567E-05
13	WAA	1000	0	0.0000	-0.0035	0.00001225	2.00462606	2.45567E-05
2	AAB	750	0	0.0000	-0.0035	0.00001225	1.50346955	1.84175E-05
3	AAC	500	0	0.0000	-0.0035	0.00001225	1.00231303	1.22783E-05
24	RAE	200	0	0.0000	-0.0035	0.00001225	0.40092521	4.91133E-06
6	AAF	100	0	0.0000	-0.0035	0.00001225	0.20046261	2.45567E-06
9	GAC	100	0	0.0000	-0.0035	0.00001225	0.20046261	2.45567E-06
14	WAB	1000	1	0.0010	-0.0025	0.00000625	2.00462606	1.25289E-05
15	WAC	1000	2	0.0020	-0.0015	0.00000225	2.00462606	4.51041E-06
4	AAD	500	1	0.0020	-0.0015	0.00000225	1.00231303	2.2552E-06
23	RAD	500	1	0.0020	-0.0015	0.00000225	1.00231303	2.2552E-06
17	WAD	1200	3	0.0025	-0.0010	0.000001	2.40555127	2.40555E-06
5	AAE	800	2	0.0025	-0.0010	0.000001	1.60370085	1.6037E-06
21	RAC	500	2	0.0040	0.0005	0.00000025	1.00231303	2.50578E-07
18	WAE	800	4	0.0050	0.0015	0.00000225	1.60370085	3.60833E-06
11	GAE	200	1	0.0050	0.0015	0.00000225	0.40092521	9.02082E-07
20	RAB	500	3	0.0060	0.0025	0.00000625	1.00231303	6.26446E-06
10	GAD	150	1	0.0067	0.0032	1.0028E-05	0.30069391	3.01529E-06
25	RAF	1000	7	0.0070	0.0035	0.00001225	2.00462606	2.45567E-05
19	RAA	500	4	0.0080	0.0045	0.00002025	1.00231303	2.02968E-05
7	GAA	100	1	0.0100	0.0065	0.00004225	0.20046261	8.46955E-06
28	RTB	67	1	0.0149	0.0114	0.00013054	0.13430995	1.75327E-05
8	GAB	100	2	0.0200	0.0165	0.00027225	0.20046261	5.45759E-05
12	GAF	50	1	0.0200	0.0165	0.00027225	0.1002313	2.7288E-05
26	RAG	236	5	0.0212	0.0177	0.00031281	0.47309175	0.000147988
27	RTA	117	3	0.0256	0.0221	0.00049023	0.23454125	0.000114978
	Total	12970	45	0.0035		0.0016746	26	0.000544917
							Dispersion	2.17967E-05
							St Deviation	0.004668692
							St Error	0.000933738

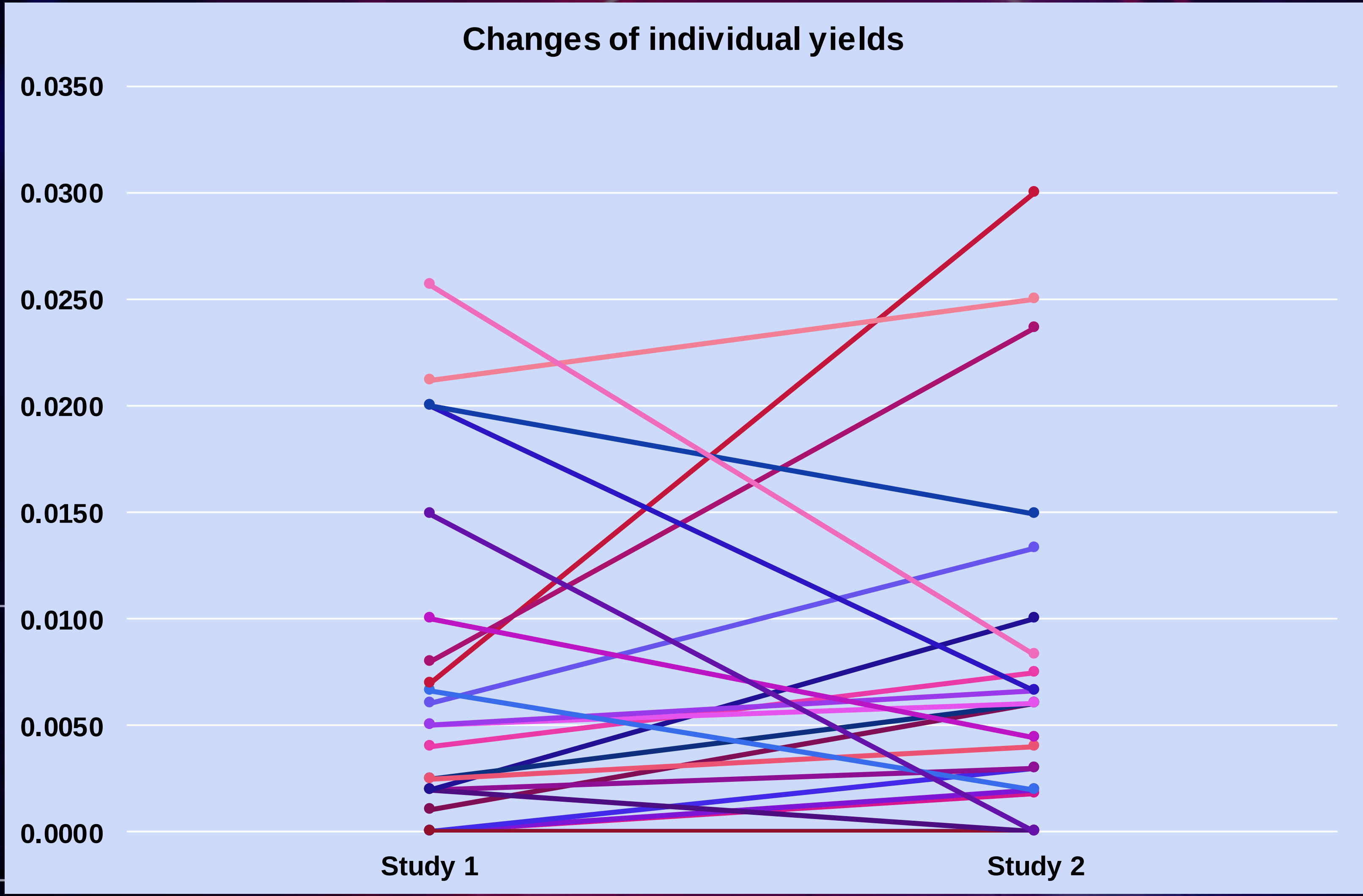
Study 2 –

The same virtual group, the same order of persons, but different numbers of cells scored and aberrations found.

Weighted group mean yield of aberrations (Dic+CR ± SE) = 0.00515±0.00109 per cell; dispersion σ^2 = 0.0000323

Patients Individual code	Cells scored	Aberrations found	Yield	Difference with mean	Squared difference	Weight, f_i	Squared difference x Weight
1AAA	1000	0	0.0000	-0.0052	2.7E-05	2.0046	0.000054
13WAA	557	1	0.0018	-0.0034	1.16E-05	1.1166	0.000013
2AAB	250	0	0.0000	-0.0052	2.7E-05	0.5012	0.000014
3AAC	513	1	0.0019	-0.0033	1.06E-05	1.0284	0.000011
24RAE	333	1	0.0030	-0.0022	4.83E-06	0.6675	0.000003
6AAF	204	0	0.0000	-0.0052	2.7E-05	0.4089	0.000011
9GAC	200	0	0.0000	-0.0052	2.7E-05	0.4009	0.000011
14WAB	500	3	0.0060	0.0008	6.4E-07	1.0023	0.000001
15WAC	1000	3	0.0030	-0.0022	4.84E-06	2.0046	0.000010
4AAD	750	0	0.0000	-0.0052	2.7E-05	1.5035	0.000041
23RAD	200	2	0.0100	0.0048	2.3E-05	0.4009	0.000009
17WAD	1000	6	0.0060	0.0008	6.4E-07	2.0046	0.000001
5AAE	500	2	0.0040	-0.0012	1.44E-06	1.0023	0.000001
21RAC	400	3	0.0075	0.0023	5.29E-06	0.8019	0.000004
18WAE	1000	6	0.0060	0.0008	6.4E-07	2.0046	0.000001
11GAE	600	4	0.0067	0.0015	2.15E-06	1.2028	0.000003
20RAB	300	4	0.0133	0.0081	6.62E-05	0.6014	0.000040
10GAD	1000	2	0.0020	-0.0032	1.02E-05	2.0046	0.000021
25RAF	200	6	0.0300	0.0248	0.000615	0.4009	0.000247
19RAA	127	3	0.0236	0.0184	0.000339	0.2546	0.000086
7GAA	450	2	0.0044	-0.0008	5.71E-07	0.9021	0.000001
28RTB	200	0	0.0000	-0.0052	2.7E-05	0.4009	0.000011
8GAB	300	2	0.0067	0.0015	2.15E-06	0.6014	0.000001
12GAF	336	5	0.0149	0.0097	9.37E-05	0.6736	0.000063
26RAG	80	2	0.0250	0.0198	0.000392	0.1604	0.000063
27RTA	1200	9	0.0083	0.0031	9.82E-06	2.4056	0.000024
Total	13200	68	0.00515			26	0.000743
						Dispersion	0.0000297
						St Deviation	0.005453
						St Error	0.001091

Individual changes of the aberration yields: Study 1 and Study 2



Calculations of the difference between weighted aberration yields: Study 2 - Study 1

Patients	Individual code	Study 1					Study 2					Difference		
Patients	Individual code	Cells scored	Aberrations found	Yield	Weight, f_i	Y*Weight	Cells scored	Aberrations found	Yield	Weight, f_i	Y*Weight	$d_i = Y_{2w} - Y_{1w}$	$d_i - d_{mean}$	$(d_i - d_{mean})^2$
1	AAA	1000	0	0.0000	2.0046	0.00000	1000	0	0.0000	1.9697	0.00000	0.00000	-0.00168	0.00000282
13	WAA	1000	0	0.0000	2.0046	0.00000	557	1	0.0018	1.0971	0.00197	0.00197	0.00029	0.00000008
2	AAB	750	0	0.0000	1.5035	0.00000	250	0	0.0000	0.4924	0.00000	0.00000	-0.00168	0.00000282
3	AAC	500	0	0.0000	1.0023	0.00000	513	1	0.0019	1.0105	0.00197	0.00197	0.00029	0.00000008
24	RAE	200	0	0.0000	0.4009	0.00000	333	1	0.0030	0.6559	0.00197	0.00197	0.00029	0.00000008
6	AAF	100	0	0.0000	0.2005	0.00000	204	0	0.0000	0.4018	0.00000	0.00000	-0.00168	0.00000282
9	GAC	100	0	0.0000	0.2005	0.00000	200	0	0.0000	0.3939	0.00000	0.00000	-0.00168	0.00000282
14	WAB	1000	1	0.0010	2.0046	0.00200	500	3	0.0060	0.9848	0.00591	0.00390	0.00222	0.00000495
15	WAC	1000	2	0.0020	2.0046	0.00401	1000	3	0.0030	1.9697	0.00591	0.00190	0.00022	0.00000005
4	AAD	500	1	0.0020	1.0023	0.00200	750	0	0.0000	1.4773	0.00000	-0.00200	-0.00368	0.00001358
23	RAD	500	1	0.0020	1.0023	0.00200	200	2	0.0100	0.3939	0.00394	0.00193	0.00025	0.00000006
17	WAD	1200	3	0.0025	2.4056	0.00601	1000	6	0.0060	1.9697	0.01182	0.00580	0.00412	0.00001701
5	AAE	800	2	0.0025	1.6037	0.00401	500	2	0.0040	0.9848	0.00394	-0.00007	-0.00175	0.00000306
21	RAC	500	2	0.0040	1.0023	0.00401	400	3	0.0075	0.7879	0.00591	0.00190	0.00022	0.00000005
18	WAE	800	4	0.0050	1.6037	0.00802	1000	6	0.0060	1.9697	0.01182	0.00380	0.00212	0.00000449
11	GAE	200	1	0.0050	0.4009	0.00200	600	4	0.0067	1.1818	0.00788	0.00587	0.00419	0.00001759
20	RAB	500	3	0.0060	1.0023	0.00601	300	4	0.0133	0.5909	0.00788	0.00186	0.00018	0.00000003
10	GAD	150	1	0.0067	0.3007	0.00200	1000	2	0.0020	1.9697	0.00394	0.00193	0.00025	0.00000006
25	RAF	1000	7	0.0070	2.0046	0.01403	200	6	0.0300	0.3939	0.01182	-0.00221	-0.00389	0.00001516
19	RAA	500	4	0.0080	1.0023	0.00802	127	3	0.0236	0.2502	0.00591	-0.00211	-0.00379	0.00001436
7	GAA	100	1	0.0100	0.2005	0.00200	450	2	0.0044	0.8864	0.00394	0.00193	0.00025	0.00000006
28	RTB	67	1	0.0149	0.1343	0.00200	200	0	0.0000	0.3939	0.00000	-0.00200	-0.00368	0.00001358
8	GAB	100	2	0.0200	0.2005	0.00401	300	2	0.0067	0.5909	0.00394	-0.00007	-0.00175	0.00000306
12	GAF	50	1	0.0200	0.1002	0.00200	336	5	0.0149	0.6618	0.00985	0.00784	0.00616	0.00003799
26	RAG	236	5	0.0212	0.4731	0.01002	80	2	0.0250	0.1576	0.00394	-0.00608	-0.00776	0.00006028
27	RTA	117	3	0.0256	0.2345	0.00601	1200	10	0.0083	2.3636	0.01970	0.01368	0.01200	0.00014407
	Total	12970	45	0.0035	26	0.09021	13200	68	0.0052	26	0.13394	0.04373		0.00036105
					Yield check	0.00347				Yield check	0.00515	Mean difference = $\Sigma d_i / n$ = 0.00168		$\Sigma(d_i - d_{mean})^2 / n(n-1)$ = 0.00000056
													Error of the difference =√ … = 0.000745296	

In this example: $t = 0.00168 / 0.000745 = 2.255$

$k = n - 1 = 25$; $t_{st} = 2.06$ *for* $p<0.05$; $t > t_{st}$ *at* $p<0.05$ (p=0.0166 by the function F.DIST.RT in MS Excel)

Comparisons between individual cases (*equal numbers of cells scored !!!*)

Patients	Cells scored	Aberration per cell distribution						ΣAbs	Aberration yield ± SE	σ^2	Aberrant cells	Ab. Cell yield ± SE
		0	1	2	3	4	5					
16FPA	200	161	25	11	2	1	0	57	**0.2850 ± 0.0468**	**0.4360**	39	**0.1950 ± 0.0281**
22FBP	200	140	52	7	1	0	0	69	**0.3450 ± 0.0406**	**0.3276**	60	**0.3000 ± 0.0325**

Student's t-test for independent groups of equal size ($n_1 = n_2$)

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s_d} = \frac{d}{s_d}$$

$$s_d = \sqrt{SE_1^2 + SE_2^2}$$

Mean yields of aberrant cells:

$d = 0.105$; $s_d = 0.0430$; $t = 2.444$

For $k = 200 + 200 - 2 = 398$ $t_{st} = 1.97$ at $p<0.05$

In this example $t > t_{st}$; $p<0.05$

(p=0.0075 by the function T.DIST.RT in MS Excel)

The result is the same if the formula for qualitative data is applied.

Mean yields of aberrations:

$d = 0.060$; $s_d = 0.06196$; $t = 0.968$

For $k = 200 + 200 - 2 = 398$ $t_{st} = 1.97$ at $p<0.05$

$t < t_{st}$; $p>0.05$

(p=0.167 by the function T.DIST.RT in MS Excel)

Fisher's test for of equality of dispersions:

$F = \sigma^2_1 / \sigma^2_2 = 0.4360 / 0.3276 = 1.331$

$k_1 = n_1 - 1 = 199$; $k_2 = n_2 - 1 = 199$

$F_{st} \approx 1.20$ for $p<0.05$; $F_{st} \approx 1.35$ for $p<0.01$

$F > F_{st}$; $p<0.05$

(p= 0.0222 by the function F.DIST.RT in MS Excel)

The comparison of distributions between individual cases: χ^2 test

$$\text{If } n_1 = n_2,\ \chi^2 = 4\left(\sum_{i=1}^{k} \frac{f_1^2}{f_1 + f_2}\right) - (n_1 + n_2)$$

Degrees of freedom *k* = Nr of classes – 1

Classes of Abs/Cell	16FPA	22FBP	f_1^2 (=16FPA)	$f_1 + f_2$	$f_1^2 / (f_1 + f_2)$
0	161	140	25921	301	86.1163
1	25	52	625	77	8.1169
2	11	7	121	18	6.7222
3	2	1	4	3	1.3333
4	1	0	1	1	1.0000
Total	200	200			103.2887
		$\chi^2 = 4 \times \Sigma(f_1^2 / (f_1 + f_2)) - (n_1+n_2)$			=13.1549

$22FBP=f_1^2$	$f_1 + f_2$	$f_1^2 / (f_1 + f_2)$
19600	301	65.1163
2704	77	35.1169
49	18	2.7222
1	3	0.3333
0	1	0.0000
		103.2887

χ^2 = 13.155; *k* = 5 – 1 = 4; χ^2_{st} = *11.14 for p=0.025*; χ^2_{st} =*13.28 for p=0.010*

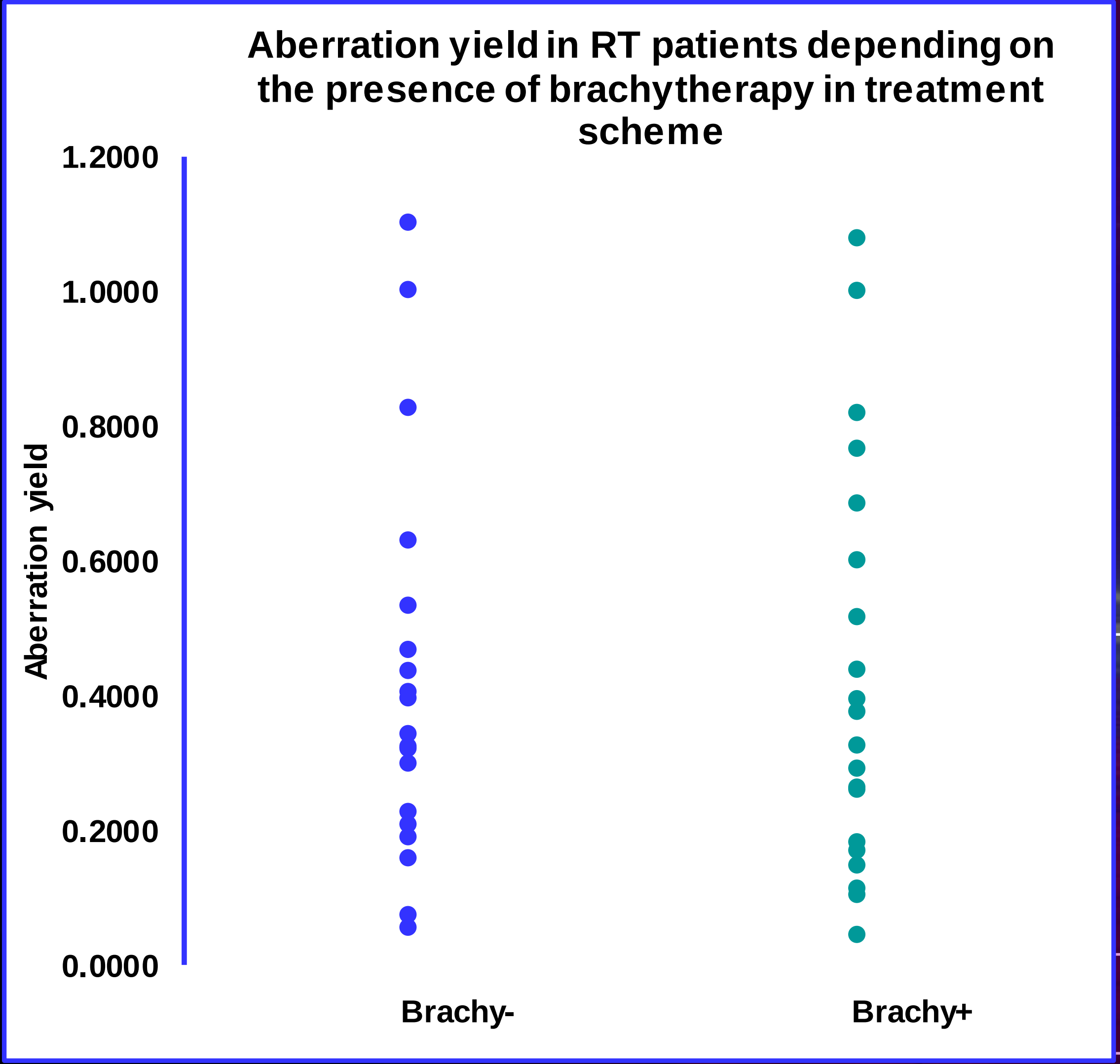
$\chi^2 > \chi^2_{st}$; *p<0.025* (p= 0.0105 by the function CHISQ.DIST.RT in MS Excel)

$$\text{If } n_1 \neq n_2;\ \chi^2 = \frac{(n_1+n_2)^2}{n_1 n_2}\left(\sum_{i=1}^{k} \frac{f_1^2}{f_1 + f_2} - \frac{n_1^2}{(n_1+n_2)}\right)$$

This formula is for comparison of two distributions of aberrations in different numbers of scored cells. Degrees of freedom:
k = Nr of classes – 1

Mann – Whitney *U* test (also called the Mann–Whitney–Wilcoxon)

Possible application: Comparison of two groups of RT patients by their biomarkers' yields



Brachytherapy-			Brachytherapy+	
Patients ID	Aberration yield		Patients ID	Aberration yield
II-3-3	0.0526		III-1-3	0.0438
IV-6-3	0.0732		III-13-2	0.1020
III-6-2	0.1551		II-3-4	0.1100
IV-15-3	0.1889		III-1-2	0.1449
IV-7-3	0.2075		III-11-2	0.1667
IV-3-3	0.2244		III-3-3	0.1806
IV-4-3	0.2976		III-18-2	0.2584
IV-20-5	0.3177		III-14-2	0.2600
IV-1-4	0.3220		II-5-3	0.2899
IV-5-3	0.3396		III-4-3	0.3252
IV-21-5	0.3929		III-2-3	0.3739
II-1-4	0.4030		III-9-2	0.3924
IV-18-2	0.4348		II-4-2	0.4364
IV-17-2	0.4667		III-16-2	0.5143
IV-19-5	0.5333		III-17-2	0.6000
II-2-3	0.6282		III-12-2	0.6846
IV-2-3	0.8261		III-6-3	0.7647
IV-16-2	1.0000		III-5-3	0.8182
IV-8-3	1.1011		III-10-2	1.0000
			III-15-2	1.0769

$n_1 = 19$ $n_2 = 20$

Mann – Whitney *U* test: Algorithm

1. Put the observations (e.g., aberration yields) from both groups into one set and sort them in ascending order.
2. Assign numeric ranks to all the observations (e.g., aberration yields), beginning with 1 for the smallest value. Where there are groups of tied values, assign a rank equal to the midpoint of unadjusted rankings (e.g., the ranks of (3, 5, 5, 5, 5, 8) are (1, 3.5, 3.5, 3.5, 3.5, 6), where the unadjusted ranks would be (1, 2, 3, 4, 5, 6)). Total number of ranks, N, must be equal to $n_1 + n_2$.
3. Add up the ranks for the observations which came from group 1 (= R_1) and from group 2 (= R_2).
4. Calculate U values: $$U_1 = R_1 - \frac{n_1(n_1+1)}{2}$$ $$U_2 = R_2 - \frac{n_2(n_2+1)}{2}$$
5. Check for correctness, i.e. whether $$U_1 + U_2 = n_1 n_2$$
6. Choose the smaller value of U_1 and U_2 and use it to compare with tabulated values of U at certain significance thresholds: [if (saburchill.com)] or [Microsoft Word - 5.a5.07.wmw.doc (usask.ca)]
7. The difference is significant, if $U_{obtained} < U_{st}$, i.e., U must be **LOWER** than U_{st}.

Example of the application of Mann – Whitney *U* test:

comparison of two groups of RT patients by their biomarkers' yields

Patients ID	Aberration yield	Rank
III-1-3	0.0438	1
II-3-3	0.0526	2
IV-6-3	0.0732	3
III-13-2	0.1020	4
II-3-4	0.1100	5
III-1-2	0.1449	6
III-6-2	0.1551	7
III-11-2	0.1667	8
III-3-3	0.1806	9
IV-15-3	0.1889	10
IV-7-3	0.2075	11
IV-3-3	0.2244	12
III-18-2	0.2584	13
III-14-2	0.2600	14
II-5-3	0.2899	15
IV-4-3	0.2976	16
IV-20-5	0.3177	17
IV-1-4	0.3220	18
III-4-3	0.3252	19
IV-5-3	0.3396	20
III-2-3	0.3739	21
III-9-2	0.3924	22
IV-21-5	0.3929	23
II-1-4	0.4030	24
IV-18-2	0.4348	25
II-4-2	0.4364	26
IV-17-2	0.4667	27
III-16-2	0.5143	28
IV-19-5	0.5333	29
III-17-2	0.6000	30

Patients ID	Aberration yield	Rank
II-2-3	0.6282	31
III-12-2	0.6846	32
III-6-3	0.7647	33
III-5-3	0.8182	34
IV-2-3	0.8261	35
IV-16-2	1.0000	36.5
III-10-2	1.0000	36.5
III-15-2	1.0769	38
IV-8-3	1.1011	39

$R_1 = 2 + 3 + 7 + 10 + 11 + 12 + 16 + 17 + 18 + 20 + 23 + 24 + 25 + 27 + 29 + 31 + 35 + 36.5 + 39 = 385.5$

$R_2 = 1 + 4 + 5 + 6 + 8 + 9 + 13 + 14 + 15 + 19 + 21 + 22 + 26 + 28 + 30 + 32 + 33 + 34 + 36.5 + 38 = 394.5$

$U_1 = 385.5 – 19 \times 20 / 2 = 195.5$
$U_2 = 394.5 – 20 \times 21 / 2 = 184.5$

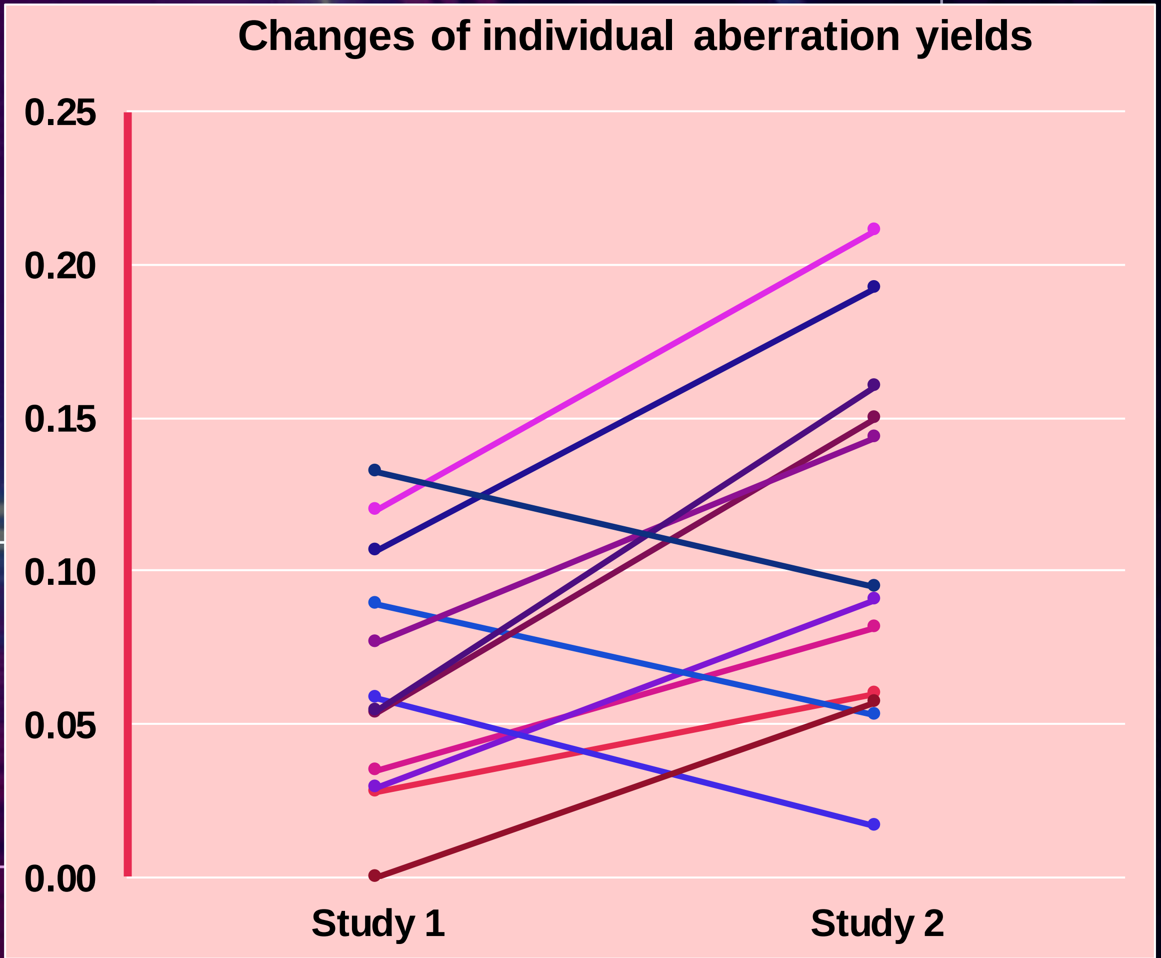
Check: $U_1 + U_2 = 195.5 + 184.5 = 380$
$n_1 \times n_2 = 19 \times 20 = 380$

For $n_1 = 19$ and $n_2 = 20$, $p=0.05$ if $U_{st} = 119$; $p=0.01$ if $U_{st} = 107$
$U = 184.5 > U_{st}$, therefore $p > 0.05$.
The groups are not statistically different for their aberration yields.

Wilcoxon signed-rank *T* test for comparison of individually matched groups

Possible application: Evaluation of the changes of biomarkers' yield in the follow-up group of RT patients

	Study 1			Study 2		
Patients	Cells	DR	Yield 1	Cells	DR	Yield 2
IV-8-2	214	6	0.028037	183	11	0.060109
IV-7-2	58	2	0.034483	320	26	0.08125
IV-3-2	25	3	0.12	109	23	0.211009
I-2-4	69	2	0.028986	695	63	0.090647
I-1-4	137	8	0.058394	603	10	0.016584
I-3-2	224	20	0.089286	133	7	0.052632
III-1-2	65	0	0	140	8	0.057143
II-2-3	75	4	0.053333	100	15	0.15
IV-4-2	65	5	0.076923	598	86	0.143813
IV-5-2	55	3	0.054545	125	20	0.16
IV-6-2	600	64	0.106667	229	44	0.19214
IV-2-2	370	49	0.132432	254	24	0.094488



Wilcoxon signed-rank *T* test: Algorithm

For a paired sample test, the data consists of samples of 2 values $(Y_1; Y_2)_i$.

1. Compute the difference $d_i = Y_2 – Y_1$ for each paired sample.
2. Take the absolute values, $| d_i |$, sort them in ascending order, and use this sorted list to assign ranks (R_1, R_2, …, R_n). The rank of the smallest observation is one, the rank of the next smallest is two, and so on. The values $d_i = 0$ should be ignored.
3. Calculate the positive-rank sum T^+ (Σ ranks of $d_i > 0$) and the negative-rank sum T^- (Σ ranks of $d_i < 0$).
4. Choose the smaller value of *T* and use it to compare with tabulated values of *T* at certain significance thresholds: Microsoft Word - WilcoxonTable2005.doc (sussex.ac.uk). In this comparison *n* is taken after subtraction of cases $d_i = 0$.
5. The difference is significant, if $T_{obtained} \le T_{st}$, i.e., *T* must be **LOWER** than T_{st}.

Example of the application of Wilcoxon signed-rank *T* test: evaluation of changes of biomarkers' yield in the group of RT patients

Patients	Yield 1	Yield 2	$d_i = Y_2 - Y_1$
IV-8-2	0.028037	0.060109	0.032072
IV-7-2	0.034483	0.08125	0.046767
IV-3-2	0.12	0.211009	0.091009
I-2-4	0.028986	0.090647	0.061662
I-1-4	0.058394	0.016584	-0.041810
I-3-2	0.089286	0.052632	-0.036654
III-1-2	0	0.057143	0.057143
II-2-3	0.053333	0.15	0.096667
IV-4-2	0.076923	0.143813	0.066890
IV-5-2	0.054545	0.16	0.105455
IV-6-2	0.106667	0.19214	0.085473
IV-2-2	0.132432	0.094488	-0.037944

Take the absolute value (module) of *di* and sort in ascending order. Assign ranks.

Patients	$\lvert d_i \rvert$	Rank
IV-8-2	0.032072	1
I-3-2	0.036654	2
IV-2-2	0.037944	3
I-1-4	0.041810	4
IV-7-2	0.046767	5
III-1-2	0.057143	6
I-2-4	0.061662	7
IV-4-2	0.066890	8
IV-6-2	0.085473	9
IV-3-2	0.091009	10
II-2-3	0.096667	11
IV-5-2	0.105455	12

Calculate T^- (Σ ranks of $d_i < 0$):
$T^- = 2 + 3 + 4 = 9$

Calculate T^+ (Σ ranks of $d_i > 0$):
$T^+ = 1 + 5 + 6 + 7 + 8 + 9 + 10 + 11 + 12 = 69$

$T_{obtained} = 9$

For n = 12, p=0.05 if $T_{st} = 12$; p=0.01 if $T_{st} = 10$.
$T_{obtained} = 9 < T_{st}$, therefore p > 0.01.
The changes of aberration yield in the studied group are statistically significant.

The comparison of qualitative (categorial) data between groups: χ^2 test, odds ratio (OR) and relative risk (RR)

Groups	Factor +	Factor -	Total
Effect +	a	b	$a+b$
Effect -	c	d	$c+d$
Total	$a+c$	$b+d$	$n=a+b+c+d$

Pearson's chi-squared test with Yates's correction for continuity:

$$\chi^2 = \frac{n\left(|ad - bc| - \frac{n}{2}\right)^2}{(a+b)(c+d)(a+c)(b+d)}$$

Degrees of freedom:

k = (number of rows – 1) x (number of columns – 1)

Odds ratio: the ratio of the odds of an event occurring in one group to the odds of it occurring in another group.

Odds of Effect$^+$ in the group "Factor $^+$" = a / c
Odds of Effect$^+$ in the group "Factor $^-$" = b / d
OR = (a/c) / (b/d) = (a x d) / (b x c).

Relative risk: the ratio of the probability of the outcome in one group to the probability of an outcome in another group.

Risk of Effect$^+$ in the group "Factor $^+$" = a / (a + c)
Risk of Effect$^+$ in the group "Factor $^-$" = b / (b + d)
RR = (a x (b + d)) / b x (a + c)

The analysis of the contingency table using χ^2 test, odds ratio and relative risk estimates is the first step in the assessment of the effect of acting factor (showing the strength of the association between the factor and the effect).

Example: **The virtual group of radiation workers created in Seminar II, split in sub-groups of monitored and non-monitored employees, to compare the numbers of individuals, who have aberration yields twice higher than mean background level, or a lower yield.**

Groups	Monitored	Non-monitored	Total
Y_{abs} > [2 x Control level]	12 = a	3 = b	15
Y_{abs} ≤ [2 x Control level]	5 = c	6 = d	11
Total	17	9	26

χ^2 test = [26 x (|12 x 6 – 3 x 5| – 26/2)^2] / [17 x 9 x 15 x 11] = 1.994
k = (number of rows – 1) x (number of columns – 1) = 1; χ^2_{st} = 3.84. χ^2 > χ^2_{st} ; p>0.05 (p = 0.158)

OR = (a/c) / (b/d) = (a x d) / (b x c) = 72 / 15 = 4.80 : 1

RR = (a x (b + d)) / b x (a + c) = 108 / 51 = 2.117

(*the statistical significance of the RR should be estimated using CL$_{95\%}$*).

The approaches to the interpretation of cytogenetic data for the assessment of the effect produced by acting factor(s), including detection versus measurement of radiation dose, will be discussed further on the next seminars.

Thank you for your attention!!!