

# Advances in Clinical and Experimental Medicine

## Guidelines for preparing a description of statistical analyses and presenting results



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## I. Statistical analysis description

All of the information regarding statistical analyses should be collected in the “Statistical analyses” section and enable a reader to repeat the analyses in exactly the same way.

### 1) Complete a list of statistical analysis tools and explain how a given tool was used, e.g.:

- a) Specify which  $\chi^2$  test type was used: a test of independence or consistency, Pearson’s  $\chi^2$  test or Maximum Likelihood, and whether Yates’s correction was used,
- b) Specify what predictor selection was used in **multiple (multivariable) regression**, and if so, how (what procedure, threshold values, etc.); also, provide the initial set of predictors,
- c) **Student’s t-test**: determine whether the test was used for matched pairs or for independent groups and whether the correction for heterogeneity of variances (Welch’s) was applied,
- d) Explain which **ANOVA** type was used (one-way, two-way, for repeated measures, etc.), and whether a variance was equal among the compared groups (then, a correction is required),
- e) The **meta-analysis** should include the following items:
  - a table with selected publications along with effects size or other measures,
  - the type of the model used (fixed or random) with the criteria for making a choice (prior analysis),
  - the summary of the effect size integration (forest plots),
  - heterogeneity analysis,
  - publication bias assessment using funnel plots (in each case), Begg’s and Mazumdar’s test or Egger’s test (when  $n > 10$ , in both cases),
- f) Statistical analysis tools available in Python or the R environment:
  - specify a library, package, etc., and give a reference,
  - provide a list of the function parameters, or a key part of the script in a supplementary file.

### 2) Checking the assumptions of the models or statistical tests used:

- a) **Student’s t-test, parametric ANOVA**: data normal distribution and variance homogeneity,
- b) **ANOVA (parametric) for repeated measurements**: data normal distribution, between-group variance and in-group variance (sphericity),
- c) **Pearson correlation**: normal data distribution, linear relationships between the variables,
- d) **All types of non-parametric correlations**: A monotonic relationship is recommended (not required). Thus, one can use a correlation on a non-monotonic relationship (including a scatter plot), but only when non-monotonicity can be neglected, which should be justified.
- e) **Linear regression**:
  - linear relationship between each predictor variable and the response variable,
  - no multicollinearity e.g., using Variance Inflation Factor,
  - homoscedasticity, i.e., the residual constant variance,
  - normal distribution of model residuals.
- f) **Logistic regression**:
  - a linear relationship between predictors and the logit of the response variable (Box–Tidwell test),
  - no multicollinearity among explanatory variables e.g., using VIF,
  - no extreme outliers (e.g., using Cook’s distance).

g) **Cox regression:**

- proportional hazards assumption,
- linearity: the log-hazard function is linearly related to all predictors,
- no multicollinearity among the predictors.

**3) Testing the normality of distributions: the choice of test depends on the sample size (N)**

N	Recommended way to test the normality of distribution
<10	Testing the normality of the distribution is uncertain because of low power of tests. In further analyses, use a non-parametric one or justify its normality based on appropriate publications.
10–50	Shapiro–Wilk test
>50	Any other methods (Lillieforce test, skewness, histogram, box plot, Q–Q Plot).

**4) Family of hypotheses: If so, apply the appropriate correction to the p-values.**

II. Data presentation in tables or graphs

**1) For normal distribution, the minimum range of statistics is the mean and standard deviation. Otherwise, the median, Q1, and Q3 quartiles should be used (quartiles only when n > 8, otherwise Min–Max range).**

III. Presentation of the results of the statistical analyses

**1) Statistical test results – general principles:**

- a) The test value (t, U, z, F, etc.), the number of degrees of freedom (df), and the statistical significance (p) with an accuracy of 3 decimal places; however, if p is less than 0.001, we use the expression “p < 0.001”, and when p is greater than 0.999, we use the expression “p > 0.999”.
- b) Present all post hoc test results, i.e., both below and above the adopted alpha level. However, consider using planned comparisons instead of only pairwise comparisons.
- c) Include effect size measures, such as coefficients in the regression model, OR, HR, AUC, differences between means, partial  $\eta^2$ , Kendall’s W value, Hedge’s g, etc., always with a confidence interval (CI).
- d) Avoid using unreliable methods, e.g., stepwise regression, selection of predictors for multiple regression by comparing the results of univariate regressions, or post hoc tests LSD (too liberal) resp. Duncan. Using such methods needs justification.
- e) Statistical significance – as p.
- f) Intervals presented as “(101.02–152.64)”.

**2) The test results should be reported following the examples below:**

- a) Student’s t-test:  $t(26) = 2.71, p = 0.004$
- b) ANOVA (overall test):  $F(2, 150) = 4.26, p = 0.076$
- c) Mann–Whitney test (Wilcoxon rank sum test):  $z = 2.54, p = 0.010$ , or  $U = 24, p = 0.008$
- d) Kruskal–Wallis test:  $H(2, 48) = 3.11, p = 0.211$
- e)  $\chi^2$  test:  $\chi^2 = 4.65, df = 1, p = 0.032$

f) Pearson's correlation:  $r = 0.62$ ,  $n = 45$ ,  $p = 0.004$

g) For multivariate regression models:

- Coefficients (B) and standardized coefficient ( $\beta$ ), their CIs, p-values, e.g.:

Variable	B	Lower 95% CI	Upper 95% CI	$\beta$	Lower 95% CI	Upper 95% CI	p
Intercept	14.7	-19.13	48.53	-	-	-	0.386
Var1	0.1	0.02	0.19	0.32	0.06	0.59	0.019
Var2	-0.2	-0.44	0.03	-0.23	-0.49	0.03	0.087

- Goodness-of-fit measures of a given model, including test value, p-value,  $R^2$ , Nagelkerke  $R^2$ , Concordance Index, etc. An example:  $F(5, 42) = 3.77$ ,  $p = 0.005$ ,  $\text{adj-}R^2 = 0.23$ .

### 3) Tables in supplementary materials

- They should be precisely titled using the names Supplementary Table 1, Supplementary Table 2, etc., with reference to any graph or tables included in the main text of the article (e.g. "Supplementary Table 1. The results of checking the normality of the data distribution (Shapiro–Wilk test) of the variables presented in Fig. 3. Explanations: W – the test value, df – number of freedom, p – statistical significance:").
- In accordance with general guidelines, they should contain only necessary content (i.e., variable names, test values, df or n, and p).
- Raw reports produced automatically by statistical programs are unacceptable** because they contain many unnecessary elements.

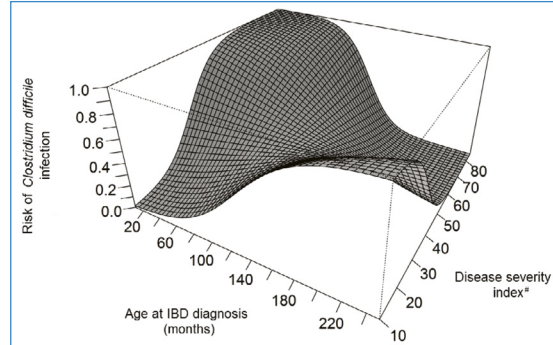
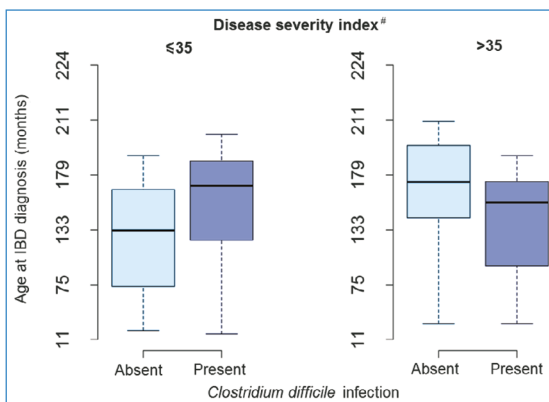
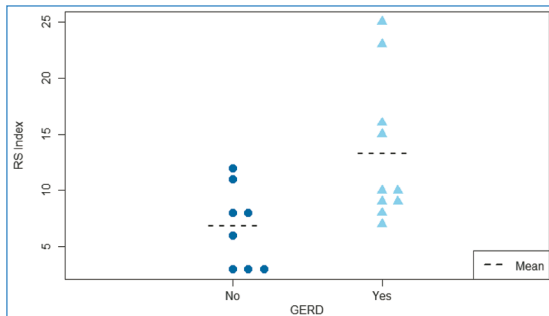
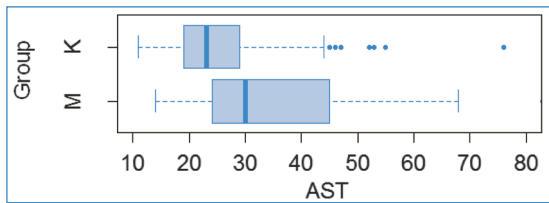
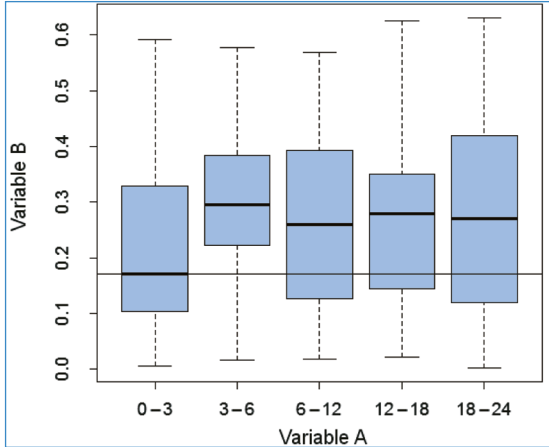
## IV. Rules for preparing figures (graphs)

**1) Figures should be titled and explained (preferably in the footer) so that they are understandable in all details without referring to other parts of the publication.**

### 2) Detailed rules:

- Font and symbol sizes should enable reading without the need to enlarge them.
- Each axis label should contain the units.
- Illustrating differences in means or other measures of central tendency:
  - Bar plots can only be used to present frequency or count data.
  - When presenting medians, Box-and-Whiskers plots should be used (composed of elements representing the median, quartiles, sample range, and outliers).
  - When presenting means, use Dot-and-Whiskers plots (presenting mean and CI) or Box-and-Whisker plots (presenting mean, SE, and CI).
  - If a sample size is less than 10, all data should be presented as dots along with the measure of central tendency appropriate for a given test.
- Regression line (e.g. in correlation), should be presented along with CI.
- If statistical significance is presented in the chart using symbols (e.g. \* as  $p < 0.05$ , etc.), in the title or explanation of the chart it is necessary to refer to tables containing all the required elements of the results of a given statistical analysis.

See next pages for examples of figures and tables.



**Table 1** Clinical characteristics of the included patients

Variable	Min	Q1	Median	Q3	Max
Age	43	65.75	68.5	74	79
BMI	18.5	20.75	22.5	23	25
CRP before	0.30	1.00	1.16	2.15	12.90
CRP after	0.31	1.00	1.33	1.98	14.92
CRP before-after	-11.90	-0.18	0.01	0.48	13.69
MMSE	12	14	19	22	23
MoCA 7.2	3	10.75	14	16.25	23

	DSM V classification					
	Mild		Moderate		Serious	
	n	%	n	%	n	%
Gender						
Men	7	35	69	35	22	18
Women	13	65	68	35	23	19

Q1, Q3—1st and 3rd quartile; \* median

**Table 1.** Characteristics of MS cases analyzed in this study.

	Genetic study		
	MS patients (336)	Male (110)	Female (226)
<b>Disease course</b>			
RR	250	76	174
SP	86	34	52
<b>Age at diagnosis</b>			
Min	23	23	23
Q1	10	13	10
Median	29	27.5	29
Q3	35	35	35
Max	50	48	50
<b>EDSS</b>			
Min	1.0	1.0	1.0
Q1	1.5	2.0	1.5
Median	3.0	3.5	3.0
Q3	5.0	6.0	4.5
Max	8.0	8.0	8.0
<b>MSSS</b>			
Min	0.38	0.38	0.45
Q1	2.65	2.82	2.60
Median	4.21	4.33	4.19
Q3	6.46	6.55	6.46
Max	9.70	9.18	9.70
<b>mRNA expression analysis</b>			
	MS patients (39)	Male (13)	Female (26)
<b>Disease course</b>			
RR	39	13	26
SP	0	0	0

RR – Relapsing-Remitting MS course. SP – Secondary Progressive MS course. EDSS – Expanded Disability Status Scale. MSSS – Multiple Sclerosis Severity Score. Min, Max – minimal and maximal value. Q1 – first quartile. Q3 – third quartile.



**Table 2** Means and standard deviations (SD) of MMSE score in AD patients ( $n=20$ ) in relation to the innate immunity measured with PBLs resistance to viral infection (viral titer express in TCID<sub>50</sub>) at  $t=0$ . ANOVA linear contrast analysis was performed to investigate the relationship between the innate immunity of PBLs (range) and effect of PRP (left table) and MMSE score (right table). In addition, the mean of the innate immunity of PBLs at  $t=0$  (before PRP treatment) and  $t=1$  (after PRP treatment) and differences between  $t=1$  and  $t=0$  are shown

PBLs resistance/level of innate immunity				MMSE**		
Range $t=0$	Mean $t=0$	Mean $t=1$	Mean difference	Mean	SD	$n$
0-1*	0.13	1.13	1.00	21.00	1.83	4
2-2.5	2.25	2.00	-0.25	18.50	4.73	4
3-3.5	3.0/	1.50	-1.5/	18.14	3./2	/
4-4.5	4.10	2.10	-2.00	14.40	2.79	5

$F_{contrast} = 12.91, df = 1;16, p = 0.0024$   
 $r_{effect\ size} = 0.66$   
 $H_0$ : mean difference is the same in all groups  
 $H_1$ : linear relation between PRP effect and level of innate immunity

$F_{contrast} = 7.67, df = 1;16, p = 0.0136$   
 $r_{effect\ size} = 0.56$   
 $H_0$ : mean MMSE is the same in all groups  
 $H_1$ : linear relation between mean MMSE and level of innate immunity

\*PBLs with complete resistance to viral infection, high level of innate immunity  
 \*\*Measured at the time of inclusion into the project  
 $t=0$  before PRP treatment  
 $t=1$  after PRP treatment

**Table 3** Spontaneous and VSV-induced cytokine production by PBLs of AD patients ( $n=20$ ) after PRP treatment. General impact and data grouped by MMSE score are present. Means of ratio of the cytokine levels (before/after PRP treatment) are presented for all levels of the MMSE score. Virus effect was considered as the ratio of VSV induced and spontaneous. ANOVA linear contrast was used to test relationship between MMSE and virus effect ( $F$ -test). PRP effect was tested with Student  $t$  test

MMSE Range	$n$	TNF- $\alpha$		IFN- $\gamma$		IL-1 $\beta$		IL-10	
		Spontaneous	VSV- induced	Spontaneous	VSV- induced	Spontaneous	VSV- induced	Spontaneous	VSV- induced
23-22	6	0.320	0.161	0.817	0.989	0.729	0.691	0.131	0.213
21-19	5	1.181	2.548	0.766	1.123	0.794	0.935	1.091	0.868
18-14	5	0.491	0.420	1.036	0.712	0.352	0.145	0.059	0.027
13-12	4	2.551	1.039	0.674	0.867	0.973	0.498	1.303	0.987
Total mean ratio after/before (regardless of MMSE)	20	0.747	0.593	0.821	0.916	0.658	0.472	0.288	0.246
Mean change [%] after PRP treatment		-25.3%	-40.7	-17.9	-8.4	-34.2	-52.8	-71.2	-75.4
CI95% for the mean change	Lower	-71.20%	-81.6	-33.8	-25.5	-54.8	-76	-89.8	-91.4
	Upper	102.60%	102.60	3.30	10.10	-5.40	-14.50	-16.90	-22.80
$F$ -test for MMSE effect		0.179		0.293		2.794		0.695	
$H_0$ : VSV effect independent of MMSE									
$H_1$ : linear relationship									
$p$ value for $F$ -test		0.678		0.596		0.114		0.417	
$r_{effect\ size}$ (MMSE effect size)		0.105		0.134		0.386		0.204	
Student $t$ test for PRP effect		-0.574	-0.819	-1.687	-0.862	-2.126	-2.322	-2.247	-2.423
$H_0$ : total mean ratio $\geq 1$									
$H_1$ : total mean ratio $< 1$									
$p$ value for the $t$ test		0.2862	0.2114	0.054	0.1996	0.0234**	0.0158**	0.0184**	0.0128**
Power of the $t$ test		0.145	0.299	0.191	0.092	0.685	0.918	0.915	0.943

$r_{effect\ size}$  means the correlation coefficient between ratio of VSV- induced and spontaneous and MMSE means both non-contrast between-group variation and the within-group variation are incorporated; \*\* statistically significant

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