

# Biostatistics 102: Quantitative Data – Parametric & Non-parametric Tests

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In this article, we are going to discuss on the statistical tests available to analyse continuous outcome variables. The parametric tests will be applied when normality (and homogeneity of variance) assumptions are satisfied otherwise the equivalent non-parametric test will be used (see table I).

**Table I. Parametric vs Non-Parametric tests.**

Parametric	Non-Parametric
1 Sample T-test	Sign Test/Wilcoxon Signed Rank test
Paired T-test	Sign Test/Wilcoxon Signed Rank test
2 Sample T-test	Mann Whitney U test/Wilcoxon Sum Rank test
ANOVA	Kruskal Wallis test

We shall look at various examples to understand when each test is being used.

## 1 SAMPLE T-TEST

The 1-Sample T test procedure determines whether the mean of a single variable differs from a specified constant. For example, we are interested to find out whether subjects with acute chest pain have abnormal systolic (normal = 120 mmHg) and/or diastolic (normal = 80 mmHg) blood pressures. 500 subjects presenting themselves to an emergency physician were enrolled.

### Assumption for 1 sample T test: Data are normally distributed.

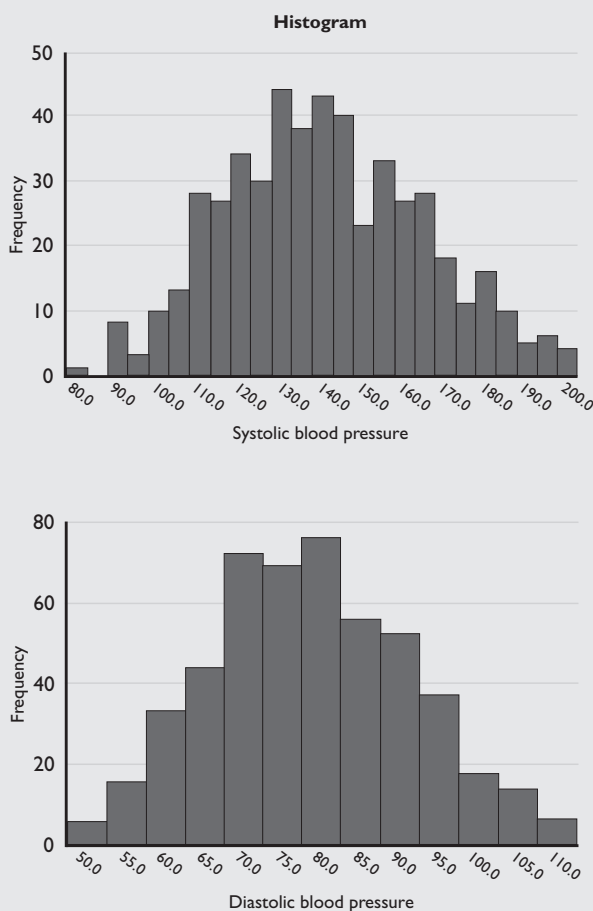
We have discussed in the last article<sup>(1)</sup> on how to check the normality assumption of a quantitative data. One issue being highlighted was that these formal normality tests are very sensitive to the sample size of the variable concerned. As seen here, table II shows that the normality assumptions for both the systolic and diastolic blood pressures are violated but basing on their histograms (see figure 1), normality assumptions are feasible.

**Table II. Formal normality tests.**

	Tests of Normality					
	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
systolic blood pressure	.049	500	.006	.990	500	.002
diastolic blood pressure	.042	500	.032	.992	500	.011

<sup>a</sup> Lilliefors Significance Correction.

**Figure 1. Histograms of Systolic & Diastolic blood pressures.**



So with the normality assumptions satisfied, we could use the 1 Sample T-test to check whether the systolic and diastolic blood pressures for these subjects are statistically different from the norms of 120 mmHg and 80 mmHg respectively.

Firstly, a simple descriptive would give us some idea, see table III.

**Table III. Descriptive statistics for the systolic & diastolic BP.**

	Mean	Std Deviation	Minimum	Maximum	Median
systolic blood pressure	140.51	24.08	79.00	200.00	139.00
diastolic blood pressure	78.65	12.91	48.00	110.00	79.00

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To perform a 1 Sample T-test, in SPSS, use *Analyze, Compare Means, One-Sample T test*. For systolic, put test value = 120 and for diastolic put test value = 80 (we have to do each test separately). Tables IV & V shows the SPSS output.

**Table IV. 1 Sample T-test for systolic BP testing at 120 mmHg.**

One-Sample Test						
Test Value = 120						
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
systolic blood pressure	19.046	499	.000	20.5080	18.3925	22.6235

**Table V. 1 Sample T-test for diastolic BP testing at 80 mmHg.**

One-Sample Test						
Test Value = 80						
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
diastolic blood pressure	-2.345	499	.019	-1.3540	-2.4886	-0.2194

These subjects had a much higher systolic BP ( $p < 0.001$ , difference = 20.5, 95% CI 18.4 to 22.6) compared to the norm of 120 mmHg. This difference is clinically 'relevant' too. For the diastolic BP, though there was a statistical significance of 1.35 (95% CI 0.22 to 2.5,  $p = 0.019$ ) lower than the norm of 80 mmHg, this difference may not be of clinical significance. By now, we should realize that the p-value is significantly affected by sample size<sup>(2)</sup>, thus we should be looking at the clinical significance first then the statistical significance.

If the normality assumptions were not satisfied, then the equivalent non-parametric Sign test or Wilcoxon Signed Rank test would be used. In SPSS, before we could perform the non-parametric analysis, we will have to create a new variable in the dataset, say, *sysnorm* (which is just a column of 120). Use the *Transform, Compute* command to do this (likewise, we have to create a new variable, say, *dianorm* which is just a column of 80). Then go to *Analyze, Non Parametric tests, 2 related samples* to do the tests (we can do both tests for systolic and diastolic simultaneously, Tables VI & VII show the SPSS outputs). In this case, we are analyzing the medians of the variables rather than their means.

**Table VI. Wilcoxon Signed Rank tests.**

Test Statistics <sup>c</sup>		
	SYSTOLIC - systolic blood pressure	DIASTOLIC - diastolic blood pressure
z	-14.965	-2.474
Asymp. Sig. (2-tailed)	.000	.013

<sup>c</sup> Wilcoxon Signed Ranks test.

**Table VII. Sign test.**

Test Statistics <sup>a</sup>		
	SYSTOLIC - systolic blood pressure	DIASTOLIC - diastolic blood pressure
z	-13.169	-2.343
Asymp. Sig. (2-tailed)	.000	.019

<sup>a</sup> Sign Test.

In the Sign test, the magnitude of the differences between the variable and the norm is not taken into consideration when deriving the significance. It uses the number of positives and negatives of the differences. Thus if there were nearly equal numbers of positives and negatives, then no statistical significance will be found regardless of the magnitude of the positives/negatives. The Wilcoxon Signed Rank test, on the other hand, uses the magnitude of the positives/negatives as ranks in the calculation of the significance, thus a more sensitive test.

## PAIRED T-TEST

When the interest is in the before and after responses of an outcome (*within group comparison*), say, the systolic BP before and after an intervention, the paired T-test would be applied.

Table VIII shows the descriptive statistics for the before and after intervention systolic BPs of 167 subjects.

**Table VIII. Descriptive statistics for the before & after intervention systolic BP.**

	Mean	Std Deviation	Minimum	Maximum	Median
systolic BP before	142.31	22.38	90.00	200.00	139.00
systolic BP after	137.14	24.87	90.00	199.00	137.00

## Assumption for the Paired T test:

*The difference between the before & after is normally distributed*

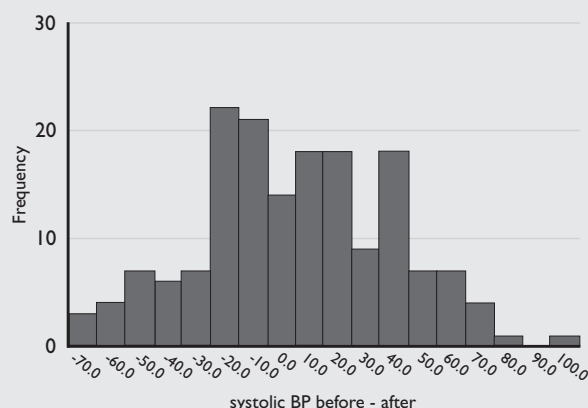
We will have to compute a new variable for the difference between the before & after systolic BP and then check its normality assumption. Table IX shows the formal tests for the checking of the normality

assumption and figure 2 shows the corresponding histogram.

**Table IX. Normality assumption checks.**

	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Systolic BP before	.048	167	.200	.991	167	.388

**Figure 2.** Histogram of the difference between the Before & After intervention systolic BP.



Since the normality assumption is satisfied, we can use the paired T-test to perform the analysis: In SPSS, use *Analyze, Compare Means, Paired Samples T test*. Table X shows the SPSS output for the paired T-test.

Clinically there was a mean reduction of 5.17 mmHg but this was not statistically significant ( $p = 0.052$ ). *Should we then increase the sample size to 'chase after the p-value'?* We shall discuss this issue at the end of this article.

Alternatively, we can use the 1-Sample T test (with test value = 0) on the difference between the Before & After to check whether there was a statistical significance; see Table XI.

In the event that the normality assumption was not satisfied, we will use the Wilcoxon Signed Rank test to perform the comparison on the medians. In SPSS, use *Analyze, Non Parametric tests, 2 related samples*: table XII shows the SPSS output.

## 2 SAMPLE T-TEST

When our interest is the *Between-Group comparison*, the 2 Sample T test would be applied. For example, we want to compare the systolic BP between the normal weight and the over-weight (a proper power analysis should be done before embarking on the study<sup>(2)</sup>). 250 subjects for each group were recruited. Table XIII gives the descriptive statistics.

### Assumptions of the 2 Sample T test:

1. *Observations are normally distributed in each population.*

**Table X. Paired T-test for the Before & After intervention systolic BP.**

	Paired Differences						Sig. (2-tailed)	
	95% Confidence Interval of the Difference							
	Mean	Std. Deviation	Std. Error	Lower	Upper	t		df
<b>Pair 1</b>								
systolic BP before	5.17	34.11	2.64	-.04	10.38	1.958	166	.052
systolic BP after								

### Pair 1

systolic BP before	5.17	34.11	2.64	-.04	10.38	1.958	.052
systolic BP after							

**Table XI. 1 Sample T test for the difference between the Before & After intervention systolic BP.**

	One-Sample Test					
	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
systolic BP before - after	1.958	166	.052	5.1677	-.0442	10.3795

**Table XII. Wilcoxon Signed Rank test on the difference on the Before & After intervention systolic BP.**

Test Statistics <sup>b</sup>	
	systolic BP after - systolic BP before
z	-1.803 <sup>a</sup>
Asymp. Sig. (2-tailed)	.071

<sup>a</sup> Based on positive ranks

<sup>b</sup> Wilcoxon Signed Ranks Test

**Table XIII. Descriptive statistics of Systolic BP by group.**

	Mean	Std Deviation	Minimum	Maximum	Median
over-weight	141.65	23.06	90.00	200.00	138.00
normal-weight	97.12	10.82	80.00	132.00	100.00

2. *Homogeneity of variance (The population variances are equal).*
3. *The 2 groups are independent random samples.*

The 3<sup>rd</sup> assumption is easily checked from the design of the experiment – each subject can only be in one of the groups or intervention. The 1<sup>st</sup> assumption of normality is also easily checked by using the *Explore* option in SPSS (with group declared in the Factor list – this will produce normality checks for each group separately). Normality assumptions must be satisfied for both groups for the 2 Sample T test to be applied. Lastly, the 2<sup>nd</sup> assumption of homogeneity of variance will be given in the 2 Sample T test analysis.

**Table XIV. 2 Sample T test.**

Independent Samples Test									
	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	131.183	.000	27.638	498	.000	44.5280	1.61111	41.36258	47.69342
Equal variances not assumed			27.638	353.465	.000	44.5280	1.61111	41.35943	47.69657

To perform a 2 Sample T test, in SPSS, use *Analyze, Compare Means, Independent Samples T-test*. Table XIV shows the SPSS output.

The Levene's Test for equality of variances checks the 2<sup>nd</sup> assumption. The Null hypothesis is: Equal Variances assumed. The Sig value (given in the 3<sup>rd</sup> column) shows that the Null hypothesis of equal variances was rejected and SPSS adjusts the results for us. In this case we have to read off the p-value (Sig 2-tailed) from the 2<sup>nd</sup> line (equal variances not assumed) rather than from the 1<sup>st</sup> line (equal variances assumed). As expected, there was a significant difference in the systolic BP between the over-weight and normal ( $p < 0.001$ , difference = 44.53, 95% CI 41.36 to 47.69 mmHg)

When normality assumptions are not satisfied for any one or both of the groups, the equivalent non-parametric Mann Whitney U/Wilcoxon Ranked Sum tests should be applied. In SPSS, use *Analyze, Non Parametric tests, 2 Independent Samples*. Table XV shows the results for the non-parametric test.

**Table XV. Mann Whitney U & Wilcoxon Rank Sum tests.**

Test Statistics <sup>a</sup>	
	BPSYS
Mann-Whitney U	1664.000
Wilcoxon W	33039.000
z	-18.454
Asymp. Sig. (2-tailed)	.000

<sup>a</sup> Grouping Variable: TRT.

Observe that only 1 p-value will be given for both Mann Whitney U and Wilcoxon Rank Sum tests.

### ANOVA (ANALYSIS OF ONE WAY VARIANCE)

The ANOVA is just an extension of the 2-Sample T test – when there are more than 2 groups to be compared. The 3 assumptions for the 2-Sample T test also apply for the ANOVA. Let's say, this time we have 3 weight groups (normal, under and over weight), the descriptive statistics is given in Table XVI.

**Table XVI. Descriptive statistics of Systolic BP by weight groups.**

	Mean	Std Deviation	Minimum	Maximum	Median
over-weight	140.89	24.65	90.00	195.00	137.50
under-weight	104.72	21.58	80.00	186.00	100.00
normal-weight	112.14	26.79	80.00	194.00	100.00

After checking for the normality assumptions, to perform an ANOVA, in SPSS, use *Analyze, Compare Means, One-Way ANOVA*. Click on *Options* and tick the *Homogeneity of Variance test*. Tables XVII & XVIII shows the results for the homogeneity of variance and ANOVA tests respectively.

**Table XVII. Homogeneity of Variance test.**

Test of Homogeneity of variances			
systolic BP			
Levene statistic	df1	df2	Sig.
4.249	2	296	.090

The Null hypothesis is: Equal Variances assumed. Since  $p = 0.09 > 0.05$ , we cannot reject the null hypothesis of equal variance.

**Table XVIII. ANOVA results.**

ANOVA					
systolic BP					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	72943.542	2	36471.771	61.126	.000
Within Groups	176613.970	296	596.669		
Total	249557.512	298			

The Null Hypothesis: All the groups' means are equal.

Since  $p < 0.001$ , not all the groups' means are equal. We would want to carry out a post-hoc test to determine where the differences were. In SPSS, under the ANOVA, click on the *Post Hoc* button and tick *Bonferroni*<sup>(3)</sup> (this method is most commonly used and rather conservative in testing for multiple

**Table XIX. ANOVA Bonferroni adjustment for multiple comparisons.**

Multiple comparisons						
Dependent variable: systolic BP Bonferroni						
(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	95% Confidence Interval		
				Sig.	Lower Bound	Upper Bound
over-weight	over-weight					
	under-weight	36.1700*	3.45447	.000	27.8528	44.4872
	normal-weight	28.7486*	3.46318	.000	20.4104	37.0868
under-weight	over-weight	-36.1700*	3.45447	.000	-44.4872	-27.8528
	under-weight					
	normal-weight	-7.4214	3.46318	.099	-15.7596	.9168
normal-weight	over-weight	-28.7486*	3.46318	.000	-37.0868	-20.4104
	under-weight	7.4214	3.46318	.099	-.9168	15.7596
	normal-weight					

\*The mean difference is significant at the .05 level.

comparisons). Table XIX shows the post-hoc multiple comparisons using Bonferroni adjustments.

The systolic BP of the over-weights were statistically (and clinically) higher than the other 2 weight groups but there was no statistical difference between the normal and under weights ( $p = 0.099$ ). **If we have carried out multiple 2 Sample T tests on our own, we have to adjust the type 1 error manually.** By Bonferroni, we have to multiply the p-value obtained by the number of comparisons performed. For 3 groups, there will be 3 comparisons (ie. A vs B, B vs C & A vs C).

Table XX shows the 2 Sample T-test between the normal and under weights. It seems that there's also a statistical difference between the 2 groups in systolic BP but taking into account multiple comparison and adjusting for type 1 error, we will have to multiply the p-value ( $= 0.033$ ) by 3 which gives the same result as in ANOVA post-hoc.

**Table XX. 2 Sample T test for Normal vs Under weight.**

Independent Samples Test						
		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
systolic BP	Equal variances assumed	7.470	.007	-2.153	197	.033
	Equal variances not assumed			-2.151	187.71	.033

When normality and homogeneity of variance assumptions are not satisfied, the equivalent non-parametric Kruskal Wallis test will be applied. In SPSS,

use *Analyze, Non Parametric tests, k Independent Samples*. Table XXI shows the SPSS results.

**Table XXI. Kruskal Wallis test on systolic BP for the 3 groups.**

Test Statistics <sup>a,b</sup>	
	systolic BP
Chi-Square	101.083
df	2
Asymp. Sig.	.000

<sup>a</sup> Kruskal Wallis Test

<sup>b</sup> Grouping Variable: GROUP

There was a statistical significant difference amongst the groups. In Kruskal Wallis, there's no post-hoc option available, we will have to do adjust for the type 1 error manually for multiple comparisons.

### TYPE I ERROR ADJUSTMENTS

A type 1 error is committed when we reject the Null Hypothesis of no difference is true. If we take the conventional level of statistical significance at 5%, it means that there is a 0.05 (5%) probability that a result as extreme as the critical value could occur just by chance, i.e. the probability of a false positive is 0.05.

There are a few scenarios when adjustments for type 1 error is required:

#### Multiple comparisons

When we are comparing between 2 treatments A & B with a 5% significance level, the chance of a true negative in this test is 0.95. But when we perform A vs B and A vs C (in a three treatment study), then the probability that neither test will give a significant result when there is no real difference is  $0.95 \times 0.95 = 0.90$ ; which means the type 1 error has increased to 10%.

Table XXII shows the probability of getting a false positive when repeated comparisons at a 5% level of significance are performed. Thus for 3 pairwise comparisons for a 3-treatment groups study (generally, number of pairwise comparisons for a n-group study is given by  $n(n-1)/2$ ), without performing a type 1 error adjustment, the probability of a false positive is 14%.

**Table XXII.**

Number of comparisons	1	2	3	4	5	6	7	8	9	10
Probability of false positive	5%	10%	14%	19%	23%	27%	30%	34%	37%	40%

As mentioned in ANOVA, Bonferroni adjustments (multiplying the p-value obtained in each multiple testing by the number of comparisons) would be the 'most convenient' and conservative test. But this test has low power (the ability to detect an existing significant difference) when the number of comparisons is 'large'. For example with 4 treatment groups, we will have 6 comparisons which means that for every pair-wise p-value obtained, we have to multiply by 6. In such a situation, other multiple comparison techniques like Tukey or Scheffe would be appropriate. Miller (1981)<sup>(4)</sup> gave a comprehensive review of the pros and cons of the various methods available for multiple comparisons. In ANOVA, this multiple comparison is automatically handled by the post-hoc option but for Kruskal Wallis test, manual adjustments needed to be carried out by the user which means that the Bonferroni method would normally be used because of its simplicity.

#### **'Chasing' after the p-value**

In the example of the Paired T-test, the before & after treatment analysis gave a p-value of 0.052 with n=167.

Perhaps this p-value will be *significant* if we increase the sample size. If the sample size was indeed increased, then the obtained p-value will have to be multiplied by 2! The reason being that we are already 'biased' by the 'positive' trend of the findings and the type 1 error needed to be controlled.

#### **Interim analysis**

Normally in large sample size clinical trials, interim analyses are carried out at certain time points to assess the efficacy of the active treatment over the control. This is carried out usually on the ethical basis that perhaps the active treatment is really superior by a larger effect difference than expected (thus a smaller sample size would be sufficient to detect a statistical significance) and we do not want to put further subjects on the control arm. These planned interim analyses with documentations of how the type 1 error adjustments for multiple comparisons must be specifically write-up in the protocol.

#### **CONCLUSIONS**

The concentration of the above discussions have been on the application of the relevant tests for different types of designs. The theoretical aspects of the various statistical techniques could be easily referenced from any statistical book.

The next article (Biostatistics 103: Qualitative Data – Test of Independence), we will discuss on the techniques available to analyse categorical variables.

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