Chapter 19
Unpaired Analysis of Variance

1 General Purpose

Unpaired t-tests are for assessing two parallel groups of similar age, gender and other characteristics treated differently. However, if you wish to compare three different treatments, three parallel groups are required, and unpaired t-tests can no longer be applied. Instead, unpaired analysis of variance (ANOVA), otherwise called one-way ANOVA must be used for analysis.

2 Schematic Overview of Type of Data File

Outcome	Treatment group (1, 2, 3, ...)
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3 Primary Scientific Question

How can one-way ANOVA evaluate the difference between three treatments.

4 Variations Expressed as the Sums of Squares

With unpaired ANOVA of 3 treatment groups variations between the data are split:

Total variation
| |
Between group variation within group variation

Variations is expressed as the sums of squares (SS) and can be added up to obtain total variation. We wish to assess whether the between-group variation is large compared to the within-group variation. This approach may be hard to understand, but the underneath simple example shows the essentials of it (n = number of patients, SD = standard deviation).

Group	n patients	mean	SD
1	n	–	–
2	n	–	–
3	n	–	–

Grand mean = (mean 1 + 2 + 3) / 3

SS beween-groups = n ($mean_1$ − grand mean)^2 + n ($mean_2$ − grand mean)^2 + ...
SS within-groups = (n−1)(SD_1 ^{2}) + (n−1) ($SD_2{}^2$) + ...

The F-test (Fisher-test) is used for testing (dfs = degrees of freedom):

$$F = \frac{\text{SS between-groups/dfs}}{\text{SS within-groups/dfs}} = \frac{\text{SS between-groups}/(3-1)}{\text{SS within-groups }/(3n-3).}$$

The F-table gives the p-value.

We should note, that, with differently sized groups, weighted grand means are required: weighted mean = (n_1 $mean_1$ + n_2 $mean_2$) / (n_1 + n_2).

5 Real Data Example

Effect of 3 compounds on Hb

Group	n patients	mean	SD
1	16	8.7125	0.8445
2	16	10.6300	1.2841
3	16	12.3000	0.9419

Grand mean = (mean 1 + 2 + 3) / 3 = 10.4926

$$\text{SS between-groups} = 16(8.7125 - 10.4926)^2 + 16(10.6300 - 10.4926)^2 \ldots.$$
$$\text{SS within-groups} = 15 \times 0.84452 + 15 \times 1.28412 + \ldots\ldots..$$
$$F = 49.9$$

df of denominator	2-tailed *P*-value	1-tailed *P*-value	Degrees of freedom (*df*) of the numerator												
			1	2	3	4	5	6	7	8	9	10	15	25	500
1	0.05	0.025	647.8	799.5	864.2	899.6	921.8	937.1	948.2	956.6	963.3	968.6	984.9	998.1	1017.0
1	0.10	0.05	161.4	199.5	215.7	224.6	230.2	234.0	236.8	238.9	240.5	241.9	245.9	249.3	254.1
2	0.05	0.025	38.51	39.00	39.17	39.25	39.30	39.33	39.36	39.37	39.39	39.40	39.43	39.46	39.50
2	0.10	0.05	18.51	19.00	19.16	19.25	19.30	19.33	19.35	19.37	19.38	19.40	19.43	19.46	19.49
3	0.05	0.025	17.44	16.04	15.44	15.10	14.88	14.73	14.62	14.54	14.47	14.42	14.25	14.12	13.91
3	0.10	0.05	10.13	9.55	9.28	9.12	9.01	8.94	8.89	8.85	8.81	8.79	8.70	8.63	8.53
4	0.05	0.025	12.22	10.65	9.98	9.60	9.36	9.20	9.07	8.98	8.90	8.84	8.66	8.50	8.27
4	0.10	0.05	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.86	5.77	5.64
5	0.05	0.025	10.01	8.43	7.76	7.39	7.15	6.98	6.85	6.76	6.68	6.62	6.43	6.27	6.03
5	0.10	0.05	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.62	4.52	4.37
6	0.05	0.025	8.81	7.26	6.60	6.23	5.99	5.82	5.70	5.60	5.52	5.46	5.27	5.11	4.86
6	0.10	0.05	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	3.94	3.83	3.68
7	0.05	0.025	8.07	6.54	5.89	5.52	5.29	5.12	4.99	4.90	4.82	4.76	4.57	4.40	4.16
7	0.10	0.05	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.51	3.40	3.24
8	0.05	0.025	7.57	6.06	5.42	5.05	4.82	4.65	4.53	4.43	4.36	4.30	4.10	3.94	3.68
8	0.10	0.05	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.22	3.11	2.94
9	0.05	0.025	7.21	5.71	5.08	4.72	4.48	4.32	4.20	4.10	4.03	3.96	3.77	3.60	3.35
9	0.10	0.05	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.01	2.89	2.72
10	0.05	0.025	6.94	5.46	4.83	4.47	4.24	4.07	3.95	3.85	3.78	3.72	3.52	3.35	3.09
10	0.10	0.05	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.85	2.73	2.55
15	0.05	0.025	6.20	4.77	4.15	3.80	3.58	3.41	3.29	3.20	3.12	3.06	2.86	2.69	2.41
15	0.10	0.05	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.40	2.28	2.08
20	0.05	0.025	5.87	4.46	3.86	3.51	3.29	3.13	3.01	2.91	2.84	2.77	2.57	2.40	2.10
20	0.10	0.05	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.20	2.07	1.86
30	0.05	0.025	5.57	4.18	3.59	3.25	3.03	2.87	2.75	2.65	2.57	2.51	2.31	2.12	1.81
30	0.10	0.05	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.01	1.88	1.64
50	0.05	0.025	5.34	3.97	3.39	3.05	2.83	2.67	2.55	2.46	2.38	2.32	2.11	1.92	1.57
50	0.10	0.05	4.03	3.18	2.79	2.56	2.40	2.29	2.20	2.13	2.07	2.03	1.87	1.73	1.46
100	0.05	0.025	5.18	3.83	3.25	2.92	2.70	2.54	2.42	2.32	2.24	2.18	1.97	1.77	1.38
100	0.10	0.05	3.94	3.09	2.70	2.46	2.31	2.19	2.10	2.03	1.97	1.93	1.77	1.62	1.31
1000	0.05	0.025	5.04	3.70	3.13	2.80	2.58	2.42	2.30	2.20	2.13	2.06	1.85	1.64	1.16
1000	0.10	0.05	3.85	3.00	2.61	2.38	2.22	2.11	2.02	1.95	1.89	1.84	1.68	1.52	1.13

This value is much larger than the critical F-value producing a $p < 0.05$, because, with 2 (numerator) and 45 (denominator) degrees of freedom the critical F-value should be between 3.32 and 3.97. The difference between the three treatment is, thus, very significant. A table of critical F-values is given on the next page. The internet provides, however, many critical F-value calculators, that are more precise.

We should add that, in case 2 groups, the F-value produced by ANOVA equals the t-test squared ($F = t^2$). T-statistics is, indeed, a simple form of analysis of variance.

6 Conclusion

The above examples show how one-way ANOVA can be used to test the significance of difference between three treatments. However, it does not tell us whether treatment 1 is better than 2, 2 better than 3, or 1 better than 3, or any combinations of these effects. For that purpose post hoc tests are required comparing the treatments one by one. Unpaired t-tests should be appropriate for the purpose.

7 Note

More background, theoretical and mathematical information of unpaired and paired ANOVA is given Statistics applied to clinical studies 5th edition, Chap. 2, Springer Heidelberg Germany, 2012, from the same authors.