# E-Learning Week: Repeated Measurements Studies

## §1. Nature and data structure of repeated measurements study

In repeated measurement studies, response to treatment is measured several times (at different time points) during or after the treatment is administered. The data for a typical treatment group has the structure as follows:

| | Time | | | | | |
|---|---|---|---|---|---|---|
| Subject | 1 | $\cdots$ | $j$ | $\cdots$ | t | Mean |
| 1 | $X_{11}^{(k)}$ | $\cdots$ | $X_{1j}^{(k)}$ | $\cdots$ | $X_{1t}^{(k)}$ | $X_{1\cdot}^{(k)}$ |
| $\vdots$ | | | | | | |
| $i$ | $X_{i1}^{(k)}$ | $\cdots$ | $X_{ij}^{(k)}$ | $\cdots$ | $X_{it}^{(k)}$ | $X_{i\cdot}^{(k)}$ |
| $\vdots$ | | | | | | |
| $n_k$ | $X_{n_k1}^{(k)}$ | $\cdots$ | $X_{n_kj}^{(k)}$ | $\cdots$ | $X_{n_kt}^{(k)}$ | $X_{n_k\cdot}^{(k)}$ |
| Mean | $X_{\cdot1}^{(k)}$ | $\cdots$ | $X_{\cdot j}^{(k)}$ | $\cdots$ | $X_{\cdot t}^{(k)}$ | $X_{\cdot\cdot}^{(k)}$ |

The data structure is the same as that of a two-factor factorial design (taking Time and Treatment as two factors). But there is an essential difference: unlike the factorial design, the columns are not independent.

There are three goals in repeated measurement studies:

1. To compare treatments with respect to their mean levels of response (treatment effect).
2. To investigate whether there is any trend over time (time effect).
3. To investigate whether there is any difference in trend among treatments (treatment by time interaction).

## §2. Analysis of variance of repeated measurements

Since the data of repeated measurements study has the same structure as that of a factorial design, the ordinary ANOVA approach can be formally applied.

The following effects (SS's) must be considered. They constitute the sources of variations in the data.

1. Treatment effect.
2. Time effect.
3. Treatment by Time interaction, since Treatment and Time are crossed and there are multiple measurements at each crossed level.
4. Subject effect (nested within Treatment).

Remark: If only a portion of the levels of a factor appear at any level of another factor, the former is said to be nested within the latter.

There are FIVE sources of variation: the four above plus random errors (residuals). The ANOVA table of a repeated measurement study data with $g$ treatment groups and $t$ measurement time points is given below:

| Source | df | SS | MS | F Ratio |
|---|---|---|---|---|
| Treatment | $g-1$ | $t\sum_k n_k(\bar{X}_{\cdot\cdot}^{(k)} - \bar{X}_{\cdot\cdot}^{(\cdot)})^2$ | GMS | $F_1 = \frac{\text{GMS}}{\text{SMS}}$ |
| Times | $t-1$ | $n.\sum_j(\bar{X}_{\cdot j}^{(\cdot)} - \bar{X}_{\cdot\cdot}^{(\cdot)})^2$ | TMS | $F_2 = \frac{\text{TMS}}{\text{RMS}}$ |
| Interaction | $(g-1)(t-1)$ | $\sum_{jk} n_k(\bar{X}_{\cdot j}^{(k)} - \bar{X}_{\cdot j}^{(\cdot)} - \bar{X}_{\cdot\cdot}^{(k)} + \bar{X}_{\cdot\cdot}^{(\cdot)})^2$ | IMS | $F_3 = \frac{\text{IMS}}{\text{RMS}}$ |
| Subject | $n. - g$ | $t\ \sum_{ik}(\bar{X}_{i\cdot}^{(k)} - \bar{X}_{\cdot\cdot}^{(k)})^2$ | SMS | |
| Residuals | $(n. - g)(t-1)$ | $\sum_{ijk}(\bar{X}_{ij}^{(k)} - \bar{X}_{i\cdot}^{(k)} - \bar{X}_{\cdot j}^{(k)} + \bar{X}_{\cdot\cdot}^{(k)})^2$ | RMS | |
| Total | $tn. - 1$ | $\sum_{ijk}(\bar{X}_{ij}^{(k)} - \bar{X}_{\cdot\cdot})^2$ | | |

**Question**: Why the denominator of $F_1$ should be SMS instead of RMS?

**Remark**: In general, the F ratios in the ANOVA table above no longer have F-distributions since the columns in the data table are not independent. But $F_1$ still follows a F-distribution. Why?

- **Comparing treatments w.r.t their mean response over time**

The ratio $F_1$ in the ANOVA table can be used to test whether there is significant difference among treatments w.r.t their mean responses. Under the null hypothesis of no difference in mean response among treatments, $F_1$ follows a $F$-distribution with df $g - 1$ and $n. - g$.

- **Analysis of time trend and interaction**

Due to non-independence between the data columns, $F_2$ and $F_3$ do not in general have $F$-distributions. These two F ratios should be compared with different distributions in different situations.

**Situation 1** : Standard deviations of the responses at all $t$ time points are equal, and all the $t(t-1)/2$ correlations between pairs of responses are equal.

In this situation, $F_2$ and $F_3$ can still be compared with the $F$-distributions having the apparent df given in the ANOVA table; that is, $F_2$ is to be compared with $F_{t-1,(t-1)(n.-g)}$ distribution, $F_3$ is to be compared with $F_{(t-1)(g-1),(t-1)(n.-g)}$ distribution.

**Situation 2** : None structure on the correlations can be assumed.

In this situation, the df $t-1$ is to be modified to $\nu = (t-1)\epsilon$ where $\epsilon$ is a function of the $t(t-1)/2$ correlations taking values between 0 and 1. The value of $\epsilon$ is determined as follows:

Let $\rho_{jj'}$ be the correlation coefficient between responses at time point $j$ and $j'$ (assumed the same for all $g$ groups). Define

$$\bar{\rho}_{j\cdot} = \frac{1}{t-1} \sum_{j' \neq j} \rho_{jj'},$$

$$\bar{\rho}_{\cdot\cdot} = \frac{1}{t} \sum_{j=1}^{t} \bar{\rho}_{j\cdot}.$$

The $\epsilon$ is determined as

$$\epsilon = \frac{(t-1)(1-\bar{\rho}_{\cdot\cdot})^2}{(t-1)(1-\bar{\rho}_{\cdot\cdot})^2 - \frac{2(t-1)}{t}\sum(\bar{\rho}_{j\cdot} - \bar{\rho}_{\cdot\cdot})^2 + \sum\sum_{j \neq j'}(\rho_{jj'} - \bar{\rho}_{\cdot\cdot})^2}.$$

$F_2$ is to be compared with $F_{\nu,\nu(n.-g)}$.

$F_3$ is to be compared with $F_{\nu(g-1),\nu(n.-g)}$.

**Situation 3** : Suppose the time points are equally spaced, and the correlation has an exponential decay, i.e., $\rho_{jj'} = \rho^{|j-j'|}$ for some $\rho$.

In this situation, the adjustment factor $\epsilon$

is obtained in a different way as follows. First the $\rho$ is estimated by the procedure described in Problem 8.4 of Fleiss. Then the $\epsilon$ value is read from Table 8.4 of Fleiss.

$F_2$ and $F_3$ are then compared with the F distributions with $(t-1)$ modified by this $\epsilon$ value.

Compared with the approach to be discussed next, the $F$-tests with modified df as described above are more powerful if the assumptions on the correlations can be confirmed.

If the assumptions are in doubt, the multivariate analysis is in order.

## §3. Multivariate analysis of repeated measurements

For each subject, the $t$ measurements can be considered as an observation on a multivariate normal vector. The multivariate analysis does not depend on the assumption of independent columns.

- **Group by time interaction**

  **Two treatment groups case**

  Let

  $$\boldsymbol{\mu}^{(k)} = (\mu_1^{(k)}, \cdots, \mu_t^{(k)})$$

  denote the vector of $t$ expected responses for treatment group $k$, $k = 1, 2$. Let

  $$\bar{\boldsymbol{X}}^{(k)} = (\bar{X}_{\cdot 1}^{(k)}, \cdots, \bar{X}_{\cdot t}^{(k)})$$

  denote the corresponding sample means.

  A particular trend can be represented by a particular contrast among the $t$ mean responses.

  The hypothesis of no group by time interaction is equivalent to the equality of all trends between the groups. All trends among the $t$ means can be represented by $t-1$ independent contrasts.

Let $C$ be a $(t-1) \times t$ matrix of independent row contrast vectors. Then the hypothesis of no interaction is equivalent to

$$H_0: \quad C(\boldsymbol{\mu}^{(1)} - \boldsymbol{\mu}^{(2)}) = 0.$$

Let $\boldsymbol{S}^{(k)}$ be the sample variance-covariance matrix of group $k$. Let $X^{(k)}$ denote the $n_k \times t$ data matrix for group $k$. Then

$$\boldsymbol{S}^{(k)} = (X^{(k)})'[I_{n_k} - \frac{\mathbf{1}_{n_k}\mathbf{1}'_{n_k}}{n_k}]X^{(k)},$$

where $I_{n_k}$ is an identity matrix of dimension $n_k$, $\mathbf{1}_{n_k}$ is a vector of 1's of dimension $n_k$. The $\boldsymbol{S}^{(k)}$ is an estimate of the variance-covariance matrix of the $t$-responses based on the data of group $k$.

Suppose the variance-covariance matrix is the same for all groups. A pooled estimate of the matrix is then

$$\bar{\boldsymbol{S}} = \frac{1}{n. - g} \sum (n_k - 1) \boldsymbol{S}^{(k)}.$$

The test statistic for testing $H_0$ above is given by

$$T^2 = \sqrt{\frac{n_1 n_2}{n_1 + n_2}} (\boldsymbol{X}^{(1)} - \boldsymbol{X}^{(2)})' C' [C \bar{\boldsymbol{S}} C']^{-1} C (\boldsymbol{X}^{(1)} - \boldsymbol{X}^{(2)}).$$

Under the normality assumption and the null hypothesis,

$$F = \frac{n_1 + n_2 - 2 - (t - 2)}{(n_1 + n_2 - 2)(t - 1)} T^2$$

follows a $F$ distribution with df $t - 1$ and $n_1 + n_2 - 2 - (t - 2) = n_1 + n_2 - t$.

## General case

In general case of more then two groups, the Wilk's $\Lambda$-test can be used to test the equivalent hypothesis:

$$H_0: \quad C\boldsymbol{\mu}^{(1)} = C\boldsymbol{\mu}^{(2)} = \cdots = C\boldsymbol{\mu}^{(g)}.$$

For details, see, e.g.,

Alvin C. Rencher (1995). *Methods of Multivariate Analysis, §6.9.* John Wiley & Sons.

- **Overall time trends**

The overall time trends are the trends of the mean responses across all groups. Let

$$\bar{\boldsymbol{X}} = \frac{1}{n.}\sum_{k=1}^{g} n_k \bar{\boldsymbol{X}}^{(k)},$$

which is the vector of sample mean responses across all groups.

The totality of trends can be represented by $t-1$ contrasts. Let $C$ be the same contrast matrix as defined above. The test statistic for the overall trends is

$$T^2 = n.\bar{\boldsymbol{X}}^{'} C^{'} [C\bar{\boldsymbol{S}}C^{'}]^{-1} C\bar{\boldsymbol{X}}.$$

Under the null hypothesis of no trends,

$$F = \frac{n. - g - (t-2)}{(n. - g)(t-1)} T^2$$

follows an F distribution with $t-1$ and $n. - g - t + 2$ degrees of freedom.

- **Trend analysis and multiple comparison**

When the overall trends are significant, it is desirable to detect particular trends such as linear or quadratic trends.

The linear, quadratic, cubic trends, etc. can be analyzed by *orthogonal polynomials*.

## An introduction to orthogonal polynomials

$t$ responses at $t$ time points can be fitted exactly by a polynomial of order $t-1$, i.e.,

$$\mu_j = b_0 + b_1 T_j + b_2 T_j^2 + \cdots + b_{t-1} T_j^{t-1}, \quad j = 1, \ldots, t.$$

The polynomial above consists of $t-1$ trends: linear, quadratic, ..., as follows

$$\begin{aligned} L &= (b_1 T_1, b_1 T_2, \ldots, b_1 T_t) \\ Q &= (b_2 T_1^2, b_2 T_2^2, \ldots, b_2 T_t^2) \\ &\cdots \end{aligned}$$

However, the components above are not orthogonal; that is, $L'Q \neq 0$, etc.

A orthogonal decomposition of the polynomial can be realized by expressing the polynomial as follows:

$$\begin{aligned}\mu_j = & \beta_0 + \beta_1(a_1 + T_j) \\ & +\beta_2(a_2 + b_2T_j + T_j^2) \\ & +\beta_3(a_3 + b_3T_j + c_3T_j^2 + T_j^3 \\ & +\cdots \\ & +\beta_{t-1}(a_{t-1} + b_{t-1}T_j + \cdots + T_j^{t-1}). \\ & j = 1, \ldots, t.\end{aligned}$$

Let

$$\begin{aligned}c_{1j} &= a_1 + b_1T_j \\ c_{2j} &= a_2 + b_2T_j + T_j^2 \\ c_{3j} &= a_3 + b_3T_j + c_3T_j^2 + T_j^3 \\ & \cdots \\ c_{t-1,j} &= a_{t-1} + b_{t-1}T_j + \cdots + T_j^{t-1} \\ \\ \boldsymbol{c}_k &= (c_{k1}, c_{k2}, \ldots, c_{kt}), \\ & \quad k = 1, \ldots, t-1.\end{aligned}$$

The coefficients $a_j, b_j, \ldots$ can be solved sequentially from the equations:

$$\begin{aligned}
&\boldsymbol{c}_1^{'}\mathbf{1} = 0;\\
&\boldsymbol{c}_2^{'}\mathbf{1} = 0, \boldsymbol{c}_2^{'}\boldsymbol{c}_1 = 0;\\
&\boldsymbol{c}_3^{'}\mathbf{1} = 0, \boldsymbol{c}_3^{'}\boldsymbol{c}_1 = 0, \boldsymbol{c}_3^{'}\boldsymbol{c}_2 = 0;\\
&\cdots
\end{aligned}$$

The vectors $\boldsymbol{c}_k, k = 1, \ldots, t-1$ are called *orthogonal polynomials*.

When $\mu_j$ is replaced by $\bar{X}_{\cdot j}$, then $T_j$ should be replaced by $n_j T_j$ in the above computation.

When all the $n_j$'s are equal and the time points $T_j$ are equally spaced, the orthogonal polynomials for $t = 3, 4$ are given below:

| | | |
|---|---|---|
| $t = 3$ | $(-1, 0, 1)$ | linear |
| | $(1, -2, 1)$ | quadratic |

$$
\begin{array}{lll}
t = 4 & (-3, -1, 1, 3) & \text{linear} \\
 & (1, -1, -1, 1) & \text{quadratic} \\
 & (-1, 3, -3, 1) & \text{cubic}
\end{array}
$$

The contrasts $\boldsymbol{c}_1^{'}\bar{\boldsymbol{X}}$, $\boldsymbol{c}_2^{'}\bar{\boldsymbol{X}}$, $\boldsymbol{c}_3^{'}\bar{\boldsymbol{X}}$, and so on, can be considered as the projection of $\bar{\boldsymbol{X}}$ onto the linear, quadratic, cubic component, and so on. If $\bar{\boldsymbol{X}}$ is completely linear (in terms of its elements) , then except $\boldsymbol{c}_1^{'}\bar{\boldsymbol{X}}$, all the other contrasts will be zero.

## Trend analysis by orthogonal polynomials

To test whether a particular trend, e.g., the linear trend is significant, the orthogonal polynomial $\boldsymbol{c}_1$ is used to form the contrast. The test statistic is given by

$$L = \frac{\sqrt{n.}\boldsymbol{c}_1^{'}\bar{\boldsymbol{X}}}{\sqrt{\boldsymbol{c}_1^{'}\bar{\boldsymbol{S}}\boldsymbol{c}_1}}.$$

The test statistic for other trends can be formed in a similar way. Individually, each such statistic follows a $t$ distribution with df $n. - g$.

If several contrasts are to be tested, multiple comparison criterion must be used.

For Tukey's, Dunnett's criteria, the second df is $n. - g - t + 2$.

For Scheffe's criterion, the critical value at level $\alpha$ is given by

$$S = \sqrt{\frac{(n. - g)(t - 1)}{n. - g - t + 2} F_{t-1, n.-g-t+2, \alpha}}.$$

Note: The above Scheffe's criterion is obtained by considering

$$\max_{\boldsymbol{c}, \boldsymbol{c}'\boldsymbol{1}=0} \frac{[\sqrt{n.}\boldsymbol{c}'\bar{\boldsymbol{X}}]^2}{\boldsymbol{c}'\bar{\boldsymbol{S}}\boldsymbol{c}}.$$

## §4 Computational issue

The ANOVA table for repeated measurements can be computed by the R function `lm` in the following steps:

**Step 1** : Fit a linear model with only the main effects of Group, Time and their interaction. The anova table of this model provides correct SS for Group, Time and their interaction. Add up all the SS in this table to get total SS.

**Step 2** : For each group, fit a model with only Subject and Time effects. Add up the SS for Subject in the separate anova tables to get the SS for Subject.

**Step 3** : Get the SS for residual by subtraction.

## §5 A case study

We consider a study comparing two groups of subjects with respect to the temperature of the forehead (in degrees Celsius) measured at 30-minute intervals. The summary data of the two groups are given below:

| Group 1 | | | | |
|---|---|---|---|---|
| | Time | | | |
| Subject | 1 | 2 | 3 | 4 |
| 1 | 30.9 | 30.7 | 30.9 | 30.9 |
| 2 | 31.9 | 31.6 | 31.6 | 31.7 |
| 3 | 31.3 | 31.1 | 31.0 | 31.3 |
| 4 | 32.1 | 31.0 | 31.7 | 31.3 |
| 5 | 30.9 | 31.2 | 30.5 | 30.8 |
| 6 | 31.3 | 31.7 | 31.4 | 31.2 |
| 7 | 31.3 | 31.8 | 31.8 | 31.7 |
| 8 | 32.1 | 33.0 | 31.7 | 31.5 |
| 9 | 30.3 | 30.9 | 30.8 | 30.6 |
| 10 | 32.2 | 32.1 | 32.2 | 32.4 |

| Group 2 | | | | |
|---|---|---|---|---|
| | Time | | | |
| Subject | 1 | 2 | 3 | 4 |
| 1 | 31.5 | 30.6 | 30.8 | 31.0 |
| 2 | 31.2 | 31.2 | 31.1 | 31.3 |
| 3 | 31.3 | 31.3 | 31.5 | 31.4 |
| 4 | 30.4 | 30.8 | 30.4 | 30.2 |
| 5 | 30.7 | 30.9 | 30.9 | 30.9 |
| 6 | 29.8 | 30.8 | 30.9 | 30.8 |
| 7 | 31.4 | 32.0 | 31.7 | 31.6 |
| 8 | 30.9 | 32.4 | 31.8 | 31.9 |
| 9 | 31.1 | 31.3 | 31.2 | 31.2 |
| 10 | 31.5 | 31.5 | 31.6 | 31.7 |

**Exercise**: Study the R-code for the notes and use the code to compute:

1. The pooled estimate of the variance-covariance matrix $\bar{\boldsymbol{S}}$.
2. The pooled estimate of the correlation matrix $R$.
3. The ANOVA table.
4. Test statistic for group by time interaction.
5. Test statistic for overall time trends.

6. The three contrasts corresponding to linear, quadratic and cubic polynomials.