

## 8. The crossover study

### §8.1. Issues on crossover design

- **What is crossover design?**

A crossover design is a scheme such that different treatments are applied to the same patient sequentially over time.

E.g., in a study for the comparison of three treatments A, B and C, for a particular patient, the treatments are applied in the order B-C-A sequentially.

To maintain the comparability of the treatments, as many different orders of the treatments as possible must be considered. Some balance among the orders must be achieved. The number of patients administered with different orders should be made as equal as pos-

sible.

E.g., with three treatments, the ideal scheme is to apply all the following orders with equal number of patients for each order: A-B-C, A-C-B, B-A-C, B-C-A, C-A-B, C-B-A.

A washout period should be imposed in between any two subsequent treatments to avoid the carry-over of the effect of the previous treatment to the subsequent period.

Crossover design is only applicable to diseases which are:

- chronic,
- effect of treatments can be measured after a short period of administration,
- effect of any treatment does not last too long ( it is possible to have a short washout

period).

- **Pros and cons of crossover design**

In crossover study, each patient serves as his or her own control. This effectively controls all prognostic variables associated with the patients.

The major pitfall of crossover designs is that the carry-over effects, if not effectively washed out in the washout period, may either ruin the whole study if the data is not analyzed with caution or waste the resource put into the study.

## §8.2. Two period crossover study

- **Replication of  $2 \times 2$  Latin squares**

The data layout of a single square:

Patient	Period		Sum	Difference
	1	2		
1	A( $X_1$ )	B( $X_2$ )	$T_1 = X_1 + X_2$	$D_1 = X_1 - X_2$
2	B( $Y_1$ )	A( $Y_2$ )	$T_2 = Y_1 + Y_2$	$D_2 = Y_1 - Y_2$

The data layout of an example:

Square	Patient	Period		Sum	Difference
		1	2		
1	1	5.1	3.8	$T_{11} = 8.9$	$D_{11} = 1.3$
	2	2.9	3.9	$T_{12} = 6.8$	$D_{12} = -1.0$
2	1	0.6	1.0	$T_{21} = 1.6$	$D_{21} = -0.4$
	2	2.9	3.9	$T_{22} = 3.9$	$D_{22} = -0.7$
3	1	4.8	3.1	$T_{31} = 7.9$	$D_{31} = 1.7$
	2	4.0	5.8	$T_{32} = 9.8$	$D_{32} = -1.8$
4	1	4.4	4.9	$T_{41} = 9.3$	$D_{41} = -0.5$
	2	1.6	0.8	$T_{42} = 2.4$	$D_{42} = 0.8$
5	1	2.3	1.3	$T_{51} = 3.6$	$D_{51} = 1.0$
	2	4.1	4.7	$T_{52} = 8.8$	$D_{52} = -0.6$
6	1	4.9	2.3	$T_{61} = 7.2$	$D_{61} = 2.6$
	2	3.2	0.9	$T_{62} = 4.1$	$D_{62} = 2.3$
7	1	6.8	4.5	$T_{71} = 11.3$	$D_{71} = 2.3$
	2	2.3	4.0	$T_{72} = 6.3$	$D_{72} = -1.7$
8	1	6.1	2.2	$T_{81} = 8.3$	$D_{81} = 3.9$
	2	3.4	3.6	$T_{82} = 7.0$	$D_{82} = -0.2$

# Expectation of the measurements

Notations:

$\mu$ : overall mean response;

$\pi_j$ : effect of period  $j$ ,  $j = 1, 2$ ;

$\tau_1$ : effect of treatment A,

$\tau_2$ : effect of treatment B;

$\rho_1$ : carry-over effect of treatment A,

$\rho_2$ : carry-over effect of treatment B.

Expectations:

$$E(X_1) = \mu + \pi_1 + \tau_1,$$

$$E(X_2) = \mu + \pi_2 + \tau_2 + \rho_1,$$

$$E(Y_1) = \mu + \pi_1 + \tau_2,$$

$$E(Y_2) = \mu + \pi_2 + \tau_1 + \rho_2.$$

It follows from the above expectations that

$$E(D_1) = (\pi_1 - \pi_2) + (\tau_1 - \tau_2) - \rho_1,$$

$$E(D_2) = (\pi_1 - \pi_2) + (\tau_2 - \tau_1) - \rho_2,$$

$$E(T_1) = 2\mu + (\pi_1 + \pi_2) + (\tau_1 + \tau_2) + \rho_1,$$

$$E(T_2) = 2\mu + (\pi_1 + \pi_2) + (\tau_1 + \tau_2) + \rho_2.$$

## Inference about the treatment effects

### Hypothesis testing

Assume that  $\rho_1 = \rho_2$ . Then the test statistic for testing the null hypothesis on the treatment effects  $H_0 : \tau_1 = \tau_2$  can be constructed as

$$t_\tau = \frac{\bar{D}_1 - \bar{D}_2}{s_D} \sqrt{\frac{n}{2}},$$

where  $\bar{D}_k = \frac{1}{n} \sum_{i=1}^n D_{ik}$ ,  $k = 1, 2$ , and  $s_D$  is the pooled estimate of the standard deviation

of the differences:

$$s_D^2 = \frac{1}{2}(s_{D_1}^2 + s_{D_2}^2).$$

The statistic above follows a  $t$ -distribution with df  $2(n - 1)$  under  $H_0$ .

## Confidence interval

Note that, if  $\rho_1 = \rho_2$ ,

$$E(\bar{D}_1 - \bar{D}_2) = 2(\tau_1 - \tau_2).$$

An unbiased estimate of  $\tau_1 - \tau_2$  is given by

$$\widehat{\tau_1 - \tau_2} = \frac{1}{2}(\bar{D}_1 - \bar{D}_2).$$

A  $100(1 - \alpha)\%$  confidence interval for  $\tau_1 - \tau_2$  is constructed as

$$\frac{1}{2}(\bar{D}_1 - \bar{D}_2) \pm t_{2(n-1), \alpha/2} \left( \frac{1}{2} s_D \right) \sqrt{\frac{2}{n}}.$$

## Inference about period effect

If there is no carry-over effects, i.e.,  $\rho_1 = \rho_2 = 0$ , the equality of period effects  $\pi_1 = \pi_2$  can be tested by the following test statistic

$$t_\pi = \frac{\bar{D}_1 + \bar{D}_2}{s_D} \sqrt{\frac{n}{2}},$$

The statistic above follows a  $t$ -distribution with df  $2(n - 1)$  under the hypothesis that  $\pi_1 = \pi_2$ .

### Example:

The data of the example is summarized as follows:

Order	$n$	Sums		Differences	
		Mean	sd	Mean	sd
AB	8	7.2625	3.1645	1.4875	1.4904
BA	8	6.1375	2.5309	-0.3625	1.3575

$$s_D = \sqrt{\frac{1.4904^2 + 1.3575^2}{2}} = 1.4255.$$

The two test statistics are computed as

$$t_{\tau} = \frac{1.4875 - (-0.3625)}{1.4255} \sqrt{\frac{8}{2}} = 2.60,$$
$$t_{\pi} = \frac{1.4875 + (-0.3625)}{1.4255} \sqrt{\frac{8}{2}} = 1.58.$$

The  $p$ -value at  $t_{\tau}$  is 0.021 and at  $t_{\pi}$  is 0.136.

A 95% confidence interval for  $\tau_1 - \tau_2$  is constructed as

$$\frac{1}{2}(1.4875 + 0.3635) \pm 2.145 \left( \frac{1.4255}{2} \right) \sqrt{\frac{2}{8}}$$

i.e.,

$$0.925 \pm 0.7644 = [0.161, 1.689].$$

## Issues on carry-over effect

The equality of carry-over effects,  $\rho_1 = \rho_2$ ,

can be tested by the statistic

$$t_\rho = \frac{\bar{T}_1 - \bar{T}_2}{s_T} \sqrt{\frac{n}{2}},$$

where  $\bar{T}_k = \frac{1}{n} \sum_{i=1}^n T_{ik}$ ,  $k = 1, 2$ , and  $s_T$  is the pooled estimate of the standard deviation of the differences:

$$s_T^2 = \frac{1}{2}(s_{T_1}^2 + s_{T_2}^2).$$

The statistic above follows a  $t$ -distribution with df  $2(n - 1)$  under the equality of  $\rho_1$  and  $\rho_2$ .

For the example,

$$t_\rho = \frac{7.2625 - 6.1375}{2.8659} \sqrt{\frac{8}{2}} = 0.79,$$

which is non-significant.

## Remarks

1. In the case of unequal carry-over effects,  $t_\tau$  does not provide a valid test for the treat-

ment effects, and  $\frac{1}{2}(\bar{D}_1 - \bar{D}_2)$  does not provide an unbiased estimate for  $\tau_1 - \tau_2$ .

An unbiased estimate of  $\tau_1 - \tau_2$  can be obtained as

$$\begin{aligned} & \widehat{(\tau_1 - \tau_2)}_{\text{ADJUSTED}} \\ &= \frac{1}{2}(\bar{D}_1 - \bar{D}_2) + \frac{1}{2}(\bar{T}_1 - \bar{T}_2) \\ &= \bar{X}_1 - \bar{Y}_1. \end{aligned}$$

Note that the adjusted unbiased estimate only involves the data obtained in period 1.

2. If carry-over effects are non-zero, though they might be equal,  $t_\pi$  does not provide a valid test for the period effects.
3. The analysis on treatment effect and period effect can also be done either through the ANOVA approach or the linear model approach with Latin square designs.

- **General two period crossover study**

In general, two period crossover study can be done by assigning patients at random to one of the two orders.

The analysis can be done in exactly the same way with the adjustment for unequal sample sizes.

Let the number of patients with order AB be  $n_1$  and that with order BA  $n_2$ . The adjustment is as follows.

$$s_D^2 = \frac{(n_1 - 1)s_{D_1}^2 + (n_2 - 1)s_{D_2}^2}{n_1 + n_2 - 2},$$

$$s_T^2 = \frac{(n_1 - 1)s_{T_1}^2 + (n_2 - 1)s_{T_2}^2}{n_1 + n_2 - 2},$$

$$t_{\tau} = \frac{\bar{D}_1 - \bar{D}_2}{s_D} \sqrt{\frac{n_1 n_2}{n_1 + n_2}},$$

$$t_{\pi} = \frac{\bar{D}_1 + \bar{D}_2}{s_D} \sqrt{\frac{n_1 n_2}{n_1 + n_2}},$$

$$t_{\rho} = \frac{\bar{T}_1 - \bar{T}_2}{s_T} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}.$$

- **Non-parametric analysis of two period crossover study**

In the analysis above, it is implicitly assumed that the responses are normally (or nearly normally) distributed. If the responses are distributed quite differently from the normal distribution, as an alternative, Mann-Whitney-Wilcoxon test can be used to test the various effects.

1. For testing treatment effect, i.e.,  $\tau_1 = \tau_2$ ,

the Mann-Whitney-Wilcoxon test is applied to the ranks of the samples  $D_{i1}, i = 1, \dots, n_1$ , and  $D_{i2}, i = 1, \dots, n_2$ .

2. For testing period effect, i.e.,  $\pi_1 = \pi_2$ , the Mann-Whitney-Wilcoxon test is applied to the ranks of the samples  $D_{i1}, i = 1, \dots, n_1$ , and  $-D_{i2}, i = 1, \dots, n_2$ .
3. For testing carryover effect, i.e.,  $\rho_1 = \rho_2$ , the Mann-Whitney-Wilcoxon test is applied to the ranks of the samples  $T_{i1}, i = 1, \dots, n_1$ , and  $T_{i2}, i = 1, \dots, n_2$ .

### **Example** (cont.)

The ranks of the sums and differences of the two groups of patients (AB and BA) in the example are given in the following table:

Ranks of sums				
Sequence AB		Sequence BA		
Square	Sum	Rank	Sum	Rank
1	8.9	13	6.8	7
2	1.6	1	3.9	4
3	7.9	10	9.8	15
4	9.3	14	2.4	2
5	3.6	3	8.8	12
6	7.2	9	4.1	5
7	11.3	16	6.3	6
8	8.3	11	7.0	8
Total		77	59	

Ranks of differences				
Sequence AB		Sequence BA		
Square	Difference	Rank	Difference	Rank
1	1.3	11	-1.0	3
2	-0.4	7	-0.7	4
3	1.7	12	-1.8	1
4	-0.5	6	0.8	9
5	1.0	10	-0.6	5
6	2.6	15	2.3	13.5
7	2.3	13.5	-1.7	2
8	3.9	16	-0.2	8
Total		90.5	45.5	

The  $\chi^2$  statistic for testing  $\rho_1 = \rho_2$  is computed as

$$\chi^2 = \frac{12 \times 8 \times 8 \times (77/8 - 59/8)^2}{16^2 \times 17} = 0.89.$$

The value is not significant.

The  $\chi^2$  statistic for testing  $\tau_1 = \tau_2$  is computed as

$$\chi^2 = \frac{12 \times 8 \times 8 \times (90.5/8 - 45.5/8)^2}{16^2 \times 17} = 5.5836.$$

The  $p$  value of the test is 0.018.

The conclusion is consistent with the previous analysis.

- **Two period crossover study with binary response**

If the responses are binary with category values, say good or bad, success or failure, the

data cannot be analyzed in exactly the same way as for quantitative responses.

Let the responses be denoted by + and – for the two categories. The data of a crossover study with two treatments is summarized as follows:

Sequence AB				Sequence BA			
Period II				Period II			
Period I	+	–	Total	Period I	+	–	Total
+	$n_{11}$	$n_{12}$	$n_{1.}$	+	$m_{11}$	$m_{12}$	$m_{1.}$
–	$n_{21}$	$n_{22}$	$n_{2.}$	–	$m_{21}$	$m_{22}$	$m_{2.}$
Total	$n_{.1}$	$n_{.2}$	$n_{..}$	Total	$m_{.1}$	$m_{.2}$	$m_{..}$

## Inference on carryover effect

Let  $P_1$  and  $P_2$  denote the proportions of positive responses in the first and second sequence.

$$P_1 = \frac{2n_{11} + n_{12} + n_{21}}{2n_{..}},$$

$$P_2 = \frac{2m_{11} + m_{12} + m_{21}}{2m_{..}}.$$

It can be obtained that

$$\begin{aligned}\text{Var}(P_1) &= \frac{1}{4n_{..}^3}[n_{..}(n_{11} + n_{22}) - (n_{11} - n_{22})^2], \\ \text{Var}(P_2) &= \frac{1}{4m_{..}^3}[m_{..}(m_{11} + m_{22}) - (m_{11} - m_{22})^2].\end{aligned}$$

The test statistic for testing  $\rho_1 = \rho_2$  is

$$\chi^2 = \frac{[|P_1 - P_2| - \frac{1}{4}(\frac{1}{n_{..}} + \frac{1}{m_{..}})]^2}{\text{Var}(P_1) + \text{Var}(P_2)}.$$

Under the hypothesis of  $\rho_1 = \rho_2$ , the statistic follows an asymptotic  $\chi^2$ -distribution with 1 degree of freedom.

## **Inference on treatment effect**

If the test for carryover effect is significant, the inference on treatment effect should only be based on the data in period 1. The test

statistic for testing  $H_0 : \tau_1 = \tau_2$  is given by

$$Z = \frac{|p_A - p_B| - \frac{1}{2}\left(\frac{1}{n_{..}} + \frac{1}{m_{..}}\right)}{\sqrt{\bar{p}(1 - \bar{p})}} \sqrt{\frac{n_{..}m_{..}}{n_{..} + m_{..}}},$$

where

$$p_A = \frac{n_{1.}}{n_{..}}, \quad p_B = \frac{m_{1.}}{m_{..}}, \quad \bar{p} = \frac{n_{1.} + m_{1.}}{n_{..} + m_{..}}.$$

The null hypothesis is rejected if  $Z > z_{\alpha/2}$  at level  $\alpha$ .

If the test for carryover effect is non-significant, or it can be assumed that there is no carryover effect, The test statistic for testing  $H_0 : \tau_1 = \tau_2$  is given by

$$Z = \frac{|p'_A - p'_B| - \frac{1}{2}\left(\frac{1}{n'} + \frac{1}{m'}\right)}{\sqrt{\bar{p}'(1 - \bar{p}')}} \sqrt{\frac{n'm'}{n' + m'}},$$

where

$$p'_A = \frac{n_{12}}{n'}, \quad p'_B = \frac{m_{12}}{m'}, \quad \bar{p}' = \frac{n_{12} + m_{12}}{n' + m'},$$

and  $n' = n_{12} + n_{21}$ ,  $m' = m_{12} + m_{21}$ . The null hypothesis is rejected if  $Z > z_{\alpha/2}$  at level  $\alpha$ .

Remark: In the test statistic above, the numbers  $n_{11}$  and  $n_{22}$  are not involved, since they are non-informative.

## **Analyzing binary variables using indicator functions**

Let  $x_{i1}, x_{i2}$  denote the responses of patient  $i$  with sequence AB in period 1 and 2 respectively, where the variables take value 1 if the response is positive and 0 otherwise. Similarly define the notation  $y_{i1}, y_{i2}$  for the responses of patients with sequence BA.

Then,

$$n_{11} = \sum x_{i1}x_{i2},$$

$$n_{22} = \sum (1 - x_{i1})(1 - x_{i2}),$$

$$n_{12} = \sum x_{i1}(1 - x_{i2}),$$

$$n_{21} = \sum (1 - x_{i1})x_{i2}.$$

The  $m_{ij}$ 's have the similar expressions in terms of  $y_{i1}$ 's and  $y_{i2}$ 's.

1. Using the indicator functions, the variances  $\text{Var}(P_1)$  and  $\text{Var}(P_2)$  can be easily obtained.
2. Like in the of quantitative responses, the sums and differences can be defined, and the statistics can be formed in the same way. It results in test statistics slightly different from the ones in this subsection.

### **§8.3. Crossover study with more than two treatments**

In two-period crossover studies, the treatment effect can be analyzed only when the crossover effects of the treatments are equal.

If there are more than two treatments, crossover studies can be designed such that the treatment effects can be analyzed no matter whether or not the crossover effects are equal under the assumption that the effect of any treatment can only carry over to the immediate next period.

- **Special Latin square designs for crossover study**

The proper designs for crossover studies with more than two treatments are special Latin square designs which have the property:

Each treatment follows each of others the same number of times.

Latin squares with the above property can be obtained by the following scheme:

- Let the first row of the square be

$$1, 2, g, 3, g - 1, 4, \dots$$

- Each subsequent row is obtained from the preceding one by adding 1 to each integer; the integer  $g + 1$  is replaced by 1. Thus the second and third rows are obtained as

$$2, 3, 1, 4, g, 5, \dots$$

$$3, 4, 2, 5, 1, 6, \dots$$

- The process continues until  $g$  rows are obtained.

When  $g$  is even, the above process assures that each treatment follows each of the others exactly once within the square.

When  $g$  is odd, the mirror image of the square must be used in tandem with the generated

square, then each treatment follows each of the others exactly twice within the pair of squares.

Examples of the special Latin squares:

$g = 3$ :

$$\begin{bmatrix} 1 & 2 & 3 \\ 2 & 3 & 1 \\ 3 & 1 & 2 \end{bmatrix} \quad \begin{bmatrix} 3 & 2 & 1 \\ 1 & 3 & 2 \\ 2 & 1 & 3 \end{bmatrix}$$

$g = 5$ :

$$\begin{bmatrix} 1 & 2 & 5 & 3 & 4 \\ 2 & 3 & 1 & 4 & 5 \\ 3 & 4 & 2 & 5 & 1 \\ 4 & 5 & 3 & 1 & 2 \\ 5 & 1 & 4 & 2 & 3 \end{bmatrix} \quad \begin{bmatrix} 4 & 3 & 5 & 2 & 1 \\ 5 & 4 & 1 & 3 & 2 \\ 1 & 5 & 2 & 4 & 3 \\ 2 & 1 & 3 & 5 & 4 \\ 3 & 2 & 4 & 1 & 5 \end{bmatrix}$$

Randomized squares of the desired property can be obtained from the above squares by first permuting the rows and followed by permuting the numerals.

- **Analysis of crossover study with more than two treatments**

The analysis of  $q \times g$  Latin squares for a crossover study with  $g$  treatments and  $q$  periods are described as follows.

**Summarizing quantities:**

- $T_i$ : sum of all  $qg$  responses to Treatment  $i$ ;
- $R_i$ : sum of all  $q(g - 1)$  responses in the periods immediately following Treatment  $i$ ;
- $F_i$ : sum of all  $qg$  responses for those subjects who received Treatment  $i$  in the final period;
- $P_1$ : sum of all  $qg$  responses in the first period;
- $G$ : sum of all  $qg^2$  responses in the entire study.

Let  $\tau_i$  and  $\rho_i$  be, respectively, the direct effect and carryover effect of treatment  $i$  subject to the constraints  $\sum \pi_i = \sum_j \tau_j = \sum_k \rho_k = 0$ .

**Unbiased estimates of  $\tau_i$  and  $\rho_j$ :**

$$\hat{\tau}_i = \frac{gT_i + g^2R_i + gF_i + gP_1 - (g+2)G}{qg(g^2 - g - 2)},$$

$$\hat{\rho}_i = \frac{(g^2 - g - 1)T_i + gR_i + F_i + P_1 - gG}{qg(g^2 - g - 2)}.$$

**Sum of squares for carryover effects:**

$$\text{CSS} = \frac{q(g^2 - g - 2)}{g} \sum \hat{\rho}_i^2.$$

**Sum of squares for treatment effects (adjusted):**

$$\text{DSS} = \frac{qg(g^2 - g - 2)}{g^2 - g - 1} \sum \hat{\tau}_i^2.$$

## Residuals sum of squares:

Residuals sum of squares is computed from the ANOVA table below:

Source	df	SS
Subjects	$qg - 1$	Standard
Periods	$g - 1$	Standard
Treatments	$g - 1$	Standard
Carryover	$g - 1$	From formula
Residuals	$(qg - 3)(g - 1)$	By subtraction
Total	$qg^2 - 1$	Standard

Note: The standard sums of squares can be computed by using the linear model approach for repeated Latin squares, treating the squares as if they are for a non-crossover study.

## Inference on carryover effects

The significance of carryover effects is tested

by the statistic

$$F_C = \frac{\text{CSS}/(g-1)}{\text{RMS}}.$$

The value of the statistic is to be compared with  $F_{g-1, (qg-3)(g-1), \alpha}$  for the test of level  $\alpha$ .

## **Inference on treatment effects**

The significance of treatment effects is tested by the statistic

$$F_D = \frac{\text{DSS}/(g-1)}{\text{RMS}}.$$

The value of the statistic is also to be compared with  $F_{g-1, (qg-3)(g-1), \alpha}$  for the test of level  $\alpha$ .

## Linear model approach:

Let the response of Subject  $l$  in period  $i$  on Treatment  $j$  when Treatment  $k$  was given in the preceding period be denoted by  $X_{ijkl}$ . It can be expressed as

$$X_{ijkl} = \mu + \pi_i + \tau_j + \rho_k + \nu_l + \epsilon_{ijkl},$$

where when  $i = 1$ ,  $\rho_k$  is taken to be  $\rho_1$ , in fact, it can be taken as any constant.

The above model can be expressed, for a individual subject, as

$$\begin{aligned} X = & \mu + \sum_{i=2}^g \pi_i p_i + \sum_{j=2}^g \tau_j t_j \\ & + \sum_{k=2}^g \rho_k c_k + \sum_{l=2}^{qg} \nu_l s_l + \epsilon, \end{aligned}$$

where the dummy variables are defined as follows:

$$\begin{aligned}
p_i &= \begin{cases} 1, & \text{if period } i, \\ 0, & \text{otherwise, } i = 2, \dots, g; \end{cases} \\
t_j &= \begin{cases} 1, & \text{if treatment } j, \\ 0, & \text{otherwise, } j = 2, \dots, g; \end{cases} \\
c_k &= \begin{cases} 1, & \text{if carryover } k, \\ 0, & \text{otherwise, } k = 2, \dots, g; \end{cases} \\
s_l &= \begin{cases} 1, & \text{if subject } l, \\ 0, & \text{otherwise, } l = 2, \dots, qg. \end{cases}
\end{aligned}$$

The inferences on the carryover effects and on the treatment effects are then boiled down to the inferences on the corresponding coefficients in the above linear model.

The tests of the overall significance of the effects and multiple comparisons can be carried out in the standard way.

- An example

Patient	Period			
	1	2	3	4
1	86 (3)	77 (4)	102 (2)	122 (1)
2	79 (4)	110 (1)	77 (3)	106 (2)
2	77 (2)	90 (3)	105 (1)	120 (4)
4	52 (1)	85 (2)	69 (4)	73 (3)
5	74 (2)	73 (4)	83 (1)	105 (3)
6	96 (4)	97 (3)	94 (2)	113 (1)
7	83 (3)	87 (1)	91 (4)	104 (2)
8	82 (1)	93 (2)	102 (3)	106 (4)
9	81 (2)	74 (4)	88 (3)	111 (1)
10	64 (3)	78 (2)	77 (1)	76 (4)
11	72 (1)	76 (3)	89 (4)	109 (2)
12	81 (4)	70 (1)	93 (2)	119 (3)
13	77 (3)	57 (1)	75 (2)	93 (4)
14	76 (2)	70 (3)	60 (4)	107 (1)
15	87 (1)	80 (4)	70 (3)	93 (2)
16	66 (4)	84 (2)	68 (1)	119 (3)
17	87 (4)	84 (2)	104 (3)	122 (1)
18	72 (1)	83 (3)	91 (2)	92 (4)
19	69 (2)	65 (1)	77 (4)	100 (3)
20	81 (3)	86 (4)	61 (1)	122 (2)

The above table gives plague scores for 20 patients in a four-period crossover study balanced for carryover effects (numbers in parentheses is the treatment number).

The summary data are as follows:

Treatment	$T_i$	$R_i$	$F_i$	$\hat{\tau}_i$	$\hat{\rho}_i$
1	1723	1372	1851	0.1900	3.6100
2	1790	1331	1763	2.6150	-0.0900
3	1764	1362	1625	1.1150	-0.8900
4	1672	1342	1710	-3.9200	-2.6300

For instance,

$$\begin{aligned}
 T1 &= 86+79+77+52+ \\
 &74+96+83+82+ \\
 &81+64+72+81+ \\
 &77+76+87+66+ \\
 &87+72+69+81 = 1723.
 \end{aligned}$$

$$\begin{aligned}
 R1 &= 77+120+85+ \\
 &105+91+93+ \\
 &76+76+93+ \\
 &75+80+119+ \\
 &83+77+122 = 1372.
 \end{aligned}$$

$$\begin{aligned}
 F1 &= 86+77+102+122+ \\
 &96+97+94+113+ \\
 &81+74+88+111+ \\
 &76+70+60+107+ \\
 &87+84+104+122 = 1851.
 \end{aligned}$$

It is computed that  $CSS = 259.3650$ ,  
 $DSS = 426.9809$ ,  $RSS = 5584.21$ .

The two F-statistics are computed as

$$F_C = \frac{259.3650/3}{5584.21/51} = 0.79,$$
$$F_D = \frac{426.9809/3}{5584.21/51} = 1.30.$$

The two F values are not significant.

The R code for implementing the linear model approach for the example are given below:

```
x11=c(86,77,102,122)
x12=c(79,110,77,106)
x13=c(77,90,105,120)
x14=c(52,85,69,73)
x1=c(x11,x12,x13,x14)
x21=c(74,73,83,105)
x22=c(96,97,94,113)
x23=c(83,87,91,104)
x24=c(82,93,102,106)
x2=c(x21,x22,x23,x24)
x31=c(81,74,88,111)
x32=c(64,78,77,76)
x33=c(72,76,89,109)
x34=c(81,70,93,119)
```

```

x3 =c(x31,x32,x33,x34)
x41=c(77,57,75,93)
x42=c(76,70,60,107)
x43=c(87,80,70,93)
x44=c(66,84,68,119)
x4=c(x41,x42,x43,x44)
x51=c(87,84,104,122)
x52=c(72,83,91,92)
x53=c(69,65,77,100)
x54=c(81,86,61,122)
x5=c(x51,x52,x53,x54)
x = c(x1,x2,x3,x4,x5)
period = rep(c(1:4),20)
subject = kronecker( c(1:20), c(1,1,1,1))
tmt = c(3,4,2,1,4,1,3,2,2,3,1,4,1,2,4,3,
        2,4,1,3,4,3,2,1,3,1,4,2,1,2,3,4,
        2,4,3,1,3,2,1,4,1,3,4,2,4,1,2,3,
        3,1,2,4,2,3,4,1,1,4,3,2,4,2,1,3,
        4,2,3,1,1,3,2,4,2,1,4,3,3,4,1,2)
carryover = c(1,3,4,2,
              1,4,1,3,
              1,2,3,1,
              1,1,2,4,
              1,2,4,1,
              1,4,3,2,
              1,3,1,4,
              1,1,2,3,
              1,2,4,3,
              1,3,2,1,
              1,1,3,4,
              1,4,1,2,

```

```

1,3,1,2,
1,2,3,4,
1,1,4,3,
1,4,2,1,
1,4,2,3,
1,1,3,2,
1,2,1,4,
1,3,4,1)
period = factor(period)
subject = factor(subject)
tmt = factor (tmt)
carryover = factor(carryover)
options(contrasts=c("contr.treatment","contr.poly"))
lm.fit = lm(x~period+tmt+subject+carryover)

b = lm.fit$coef[5:7]
v = vcov(lm.fit)[5:7,5:7]
F.D=t(b)%*%solve(v)%*%b/3

b = lm.fit$coef[27:29]
v = vcov(lm.fit)[27:29,27:29]
F.C=t(b)%*%solve(v)%*%b/3

```

The F.D, F.C are the values of the two F-statistics. It produces

(F.D, F.C) = (1.2998572 0.7895844)

The command `anova(lm.fit)` produces the following ANOVA table

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
period	3	9814.7	3271.6	29.8790	2.725e-11	***
tmt	3	397.9	132.6	1.2114	0.315022	
subject	19	5977.2	314.6	2.8731	0.001405	**
carryover	3	259.4	86.5	0.7896	0.505314	
Residuals	51	5584.2	109.5			

Note: Not all the SS are correct in the above table.