Estimation of Treatment and Carryover Effects in Optimal Cross-Over Designs for Clinical Trials

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A particular multi-period cross-over design useful in trials where observations naturally are correlated and carryover effect may not die after one period—such as thorough QT, trials on diet, asthma, and others—is shown to be useful for estimation in three practical cases. The cases are: the variance balanced estimation of direct and carryover treatment effects in presence of higher-order carryover and correlated errors using ordinary least squares method; estimation of the said treatment effects under scattered missing observations; and the interim estimation of the same for trials with early stopping rules. We compare treatment effect estimates and their variances with those given in the literature for the higher-order carryover model. We also give a numerical example demonstrating estimation in all three cases.

Key Words: Correlated errors; Equineighbored; Interim estimation; Missing.

1. Introduction

Clinical trials are conducted to identify or quantify the influence of drug treatment/s on experimental units. In this case, cross-over experiments are economical. To use a cross-over design (COD) in a clinical trial, it is better to anticipate some carryover and to accommodate it in analysis.

COD is an arrangement of an experiment in which experimental units are used repeatedly by exposing them according to a sequence of treatments over a span of time. Apparently each unit is influenced by the direct effect of the current treatment and the carryover effects of previously applied treatments. The possibility of the existence of carryover effect has led many authors to advise that a parallel design be used whenever carryover is suspected. Willan and Pater (1986) examined this advice and quantified the degree of carryover required to make the parallel design preferable in terms of the power of the test of treatment effect and precision of the estimate of treatment difference. Lehmacher (1991) indicated how the usual cross-over test has to be interpreted correctly and that its bias has two different consequences, namely a conservative or a liberal test decision if a positive or a negative carryover effect exists. According to Senn (1992), if carryover is present to any appreciable degree, then the usual statistical models provide no guaranteed protection against its effects. Laird, Skinner, and Kenward (1992), however, discussed that we can rarely rule out carryover effects on a priori grounds and because their inclusion may substantially alter the interpretation of the results, it is important to include them in the analysis.

CODs suitable for estimation of a model with first-order carryover effects have been discussed by many authors, including Grizzle (1965), Kunert (1991), Carriere and Reinsel (1993), Senn (1993), Jones and Donev...
(1996), and Jones and Kenward (2003) under different assumptions. Optimality of such CODs has been studied by Hedayat and Afsarinejad (1975, 1978), Matthews (1994), Kushner (1997a, b, 1998), Afsarinejad and Hedayat (2002), Kunert and Stufken (2002), and Hedayat and Yang (2003, 2004).

Apart from the presence of carryover effects, the errors in measurements are correlated because the number of treatments is applied and observations are made on the same unit. Correlated observations models for CODs in the presence of carryover effects were discussed by Finney (1956), Taka and Armitage (1983), Laska and Meisner (1985), and Gill and Shukla (1987). Gill (1992) found that generalized least squares estimation of direct treatment effect and first-order carryover effect for CODs under autocorrelated observation model is very difficult. An easy way out is to consider a design that is robust to correlation structure similar to Ipinyomi (1986), who introduced a class of block designs called equineighborhood block designs in which each treatment is paired with other treatments in every plot position. Martin and Eccleston (1998) used orthogonal arrays of strength two to produce binary as well as nonbinary variance-balanced repeated measurements designs (CODs) under a dependence structure and carried out estimation in the presence of first-order carryover effects.

However, in experimental studies like thorough QT studies (ICH 2005; Ring et al. 2010), diet studies, asthma studies (Brusasco et al. 2002), and so on, in which multi-period COD is profitably used, it is possible that any carryover effect does not die unexpectedly after one period as mentioned by John and Quenouille (1977, p. 198). Bose and Mukherjee (2000) constructed multi-period CODs suitable for such studies. Their designs are nonbinary and optimal for estimation of direct effects under non-additive, higher-order carryover model with correlated errors (Bose and Mukherjee 2003) for a specific correlation structure that every two observations from a unit are identically correlated. Here, nonbinary means their designs have treatment sequences containing repetition of the same treatment, which is undesirable in practice. Usually, unary and binary forms of CODs are preferred, and in particular those for which analysis is simple and versatile under violation of defined norms. Here, unary means treatment sequences are complete in treatments and binary means they are incomplete. Two more situations are most likely in clinical investigations that employ multi-period CODs. Investigators would propose to terminate a trial early as per interim analyses policy, and units may drop out or not adhere to the intervention schedule.

This article discusses the multiple benefits of a multi-period COD that has the equineighbor property, which makes the design robust to violation of ordinary least squares (OLS) estimation assumption of uncorrelated observations. We refer to this design as the equineighbored cross-over (ECO) design. An ECO design is an arrangement of an experiment in which $t$ treatment are compared using $n$ units repeatedly measured for $p$ periods such that every treatment pair occur equally often in every pair of periods; it is denoted by ECO($t$, $n$, $p$). Estimation of treatment effects is restricted to ECO($t$, $n$, $p$) design because it possesses the equineighbor property for $2 \leq p \leq t$ and it is optimal under the traditional model for $p = t$ (Hedayat and Yang 2004). A simple technique to write the plan and model under consideration here is given in Section 2. We also define the measure of efficiency of separability (ES) of direct effect from carryover effects. Variance balanced estimates of direct treatment effect and first to ($p - 2$)th order carryover effects along with variance expressions are obtained in Section 3 such that, two equally distant period observations have arbitrary but common correlation. Further, we show that estimates of direct and carryover effects are suitable for interim analysis as well as in case of missing observations. A numerical example is given in Section 4. Section 5 gives an overview of the treatment effect estimates obtained in this article and compares the estimates with those computable through OLS software under the carryover and correlated error model. Matlab code for estimation of treatment effects with variances is given in the Appendix.

2. The Carryover Model and Equineighbored COD

A COD in presence of carryover effect up to $r$th($r = 0, 1, \ldots, p - 2$) order is said to be ECO($t$, $n$, $p$) design if every treatment pair occur equally often in every pair of periods.

2.1 The Model and Efficiency of Separability of Treatment Effects

Let the model for COD in presence of correlated errors and carryover effect up to $r$th order is given by

$$
\bar{Y} = \sum_{e=0}^{r} R_{e} \gamma_{e} + P_{a} \alpha + U_{b} \beta + \varepsilon, \quad r = 0, 1, \ldots, p - 2,
$$

(1)

where $\bar{Y}$ is vector of response observations ordered as $(Y_{11}, Y_{21}, \ldots, Y_{1p}; \ldots; Y_{1n}, \ldots, Y_{pm})$, and $R_{e}$ is the observation-$e$th order carryover incidence matrix. Clearly, observations in the first period have zero carryover effects of all orders. Similarly, observations in the second period have only first-order carryover effects and no higher-order effects. In the model (1), $P$ is observation-period incidence matrix given by $J_{n, 1} \otimes I_{p}$,
Estimation of Treatment and Carryover Effects in Optimal Cross-Over Designs for Clinical Trials

\( U \) is observation-unit incidence matrix given by \( I_n \otimes J_{p,1} \), and \( \gamma_e, \alpha, \beta \) are the vectors of \( \text{eth}(e = 0, 1, \ldots, r) \) order carryover effects, period effects, and unit effects, respectively. Note that \( R_0 \) and \( \gamma_0 \) denote the observation-direct treatment incidence matrix and vector of direct treatment effects. Without loss of generality, we assumed that the general mean effect is absorbed in the unit effect. \( \varepsilon \) is vector of normally distributed errors with mean zero and variance-covariance matrix \( \sigma^2 V = \sigma^2 I_n \otimes \sum_{s=0}^{p-1} h_s Z_{ps} \). Throughout, \( J \) denotes a matrix of ones, \( I \) denotes identity matrix, \( \otimes \) denotes the Kroneker product of two matrices. The incidence matrix of ones, \( J \) denotes identity matrix, and \( \otimes \) denotes the Kroneker product of two matrices. The incidence matrices \( Z_{ps} \) are \( p \times p \) neighbor matrices where \((i, j)\)th entry is equal to 1 if \(|i - j| = s \) and 0 otherwise; \((i, j)\) means \( i \)th row and \( j \)th column. The form of \( \rho_s \) can be assumed conveniently by the experimenter, for \( s = 0, 1, \ldots, p - 1 \), which indicates correlation between \( s \)-level apart observations. Generally, it is desirable to assume that correlation falls off as the distance between periods increases. The \( s \)th level nearest neighbors of \( Y_{ij} \) are \( Y_{i-s,j} \) and \( Y_{i+s,j} \) with \( i - s \geq 1 \) and \( i + s \leq p \) for \( s = 1, 2, \ldots, p - 1 \).

In accordance with the definition of ECO(\( t, n, p \)) in the presence of carryover effects up to \( r \)th order \((r = 0, 1, \ldots, p - 2) \) order, the treatment effect incidence matrices must satisfy that

\[
R_e^x = \left( I_n \otimes Z_{(p-cy)} \right) R_e^x = x_{se} (J_t - I_t)
\]

for each \( s = 1, 2, \ldots, \), \( p - e - 1 = e = 0, 1, \ldots, r \), (2)

where the constant \( x_{se} \) is the number of times \( e \)th carryover pair \((i, j)\) are \( s \)th level nearest neighbors, \( i \neq j = 0, 1, \ldots, p - 1 \), and \( R_e^x \) denotes a submatrix of \( R_e^x \) excluding all null rows in \( R_e^x \), \( e = 0, 1, \ldots, r \).

In general,

\[
x_{se} = 2(p - s - e). \tag{3}
\]

A COD(\( t, n, p \)) and an ECO(\( t, n, p \)) in presence of carryover effects up to \( r \)th order \((r = 0, 1, \ldots, p - 2) \) order is said to be balanced for correlated observations if

\[
\text{var}(\hat{\gamma}_{ei} - \hat{\gamma}_{ej}) = \text{constant}, \quad \text{for all} \quad i \neq j = 0, 1, \ldots, t - 1; \quad e = 0, 1, \ldots, r, \tag{4}
\]

where \( V \) is as defined earlier.

It is known that when there is carryover in a design, the direct treatment effects and carryover effects are not orthogonal. Designs for such applications must be measured for its ability to separate the direct and carryover effects, because a design that is poor at separating the effects may cause a direct treatment effect to be declared significant when it may be due to a positive carryover effect. The ability of separating the effects depends upon the number of occurrences of a treatment in a period \((\lambda_o)\) and the number of ordered pair occurrences of direct and an \( i \)th order carryover treatment \((\lambda_i, i = 1, 2, \ldots, r) \). A measure of ES can be calculated for a design on the basis of observed frequencies of carryover and the expected frequencies from an independent model (Hanford 2005).

Following Hanford (2005), a formula for the ES of direct effects from all assumed carryover effects in case of ECO(\( t, n, p \)) is given by

\[
ES = \left\{ 1 - \left[ \frac{\sum_{i=1}^{r} \lambda_i}{(t-1)(\lambda_0 + (t-1) \sum_{i=1}^{r} \lambda_i)} \right] \right\} \times 100, \tag{5}
\]

where \( r, (r = 0, 1, \ldots, p - 2) \) denotes the highest order of carryover effect assumed. ES of direct effects from all assumed carryover effects can be calculated using (5) for ECO(\( t, (t - 1), p \)), \( 2 \leq p \leq t \), is given by

\[
ES = \left\{ 1 - \left[ \frac{r(2p-r-1)}{(t-1)^2(2+r(2p-r-1))} \right] \right\} \times 100. \tag{6}
\]

ES of direct from first-order carryover effect in ECO(3, 6, 3) is 59% and in case of ECO(4, 12, 3) is 73%, indicating ES increases with \( t \) for ECO(\( t, (t-1), 3 \)) as expected in practice. It is interesting to note that, a comparable design, COD(4,8,2) of Afsarinejad and Hedayat (2002) has ES 59%. In general, it is easy to see from Equation (6) that ES of direct from all assumed carryover effects in ECO(\( t, (t - 1), p \), \( p = t \)) increases with \( t \). This property indicates that design is useful for analysis under higher-order carryover model.

### 2.2 Plan of ECO(\( t, (t-1), p \)) Design

Following Hedayat and Yang (2004), since ECO(\( t, (t-1), p \)) designs are balanced uniform for \( p = t \), they are universally optimal for direct effects under uncorrelated errors. In general, they are variance balanced for estimation of direct treatment effects and carryover effects, because every pair of symbols representing direct treatment and those representing residual treatment appear equally often in \( n \) treatment sequences. For OLS estimation to be viable, the designs must be robust to correlated errors, that is, equineighborhood condition (2) must hold. The above requirements become fulfilled when all \( (t-1) \) mutually orthogonal Latin squares of order \( t \) are laid down side by side. Alternatively, the following steps obtain the plan.

1. Write down or consider a Latin square of order \( t \), with its symbols as elements of Galois Field GF(\( t \)): \( \{0, 1, x, x^2, \ldots, x^{t-2}\} \). It can be written down easily as addition table of GF(\( t \)) elements. Let the \( t \) columns form treatment sequences for \( t \) units.
(ii) Multiply the Latin square by each element \(x\), \(x^2\), \(\ldots, x^{t-2}\). Each multiplication gives new treatment sequences for additional \(t\) units.

(iii) Steps (i) and (ii) together provide plan of ECO(\(t\), \(t(t-1)\), \(t\) design.

(iv) Considering any \(p\) rows of ECO(\(t\), \(t(t-1)\), \(t\) design, provide a plan of ECO(\(t\), \(t(t-1)\), \(1 < p < t\) design.

A plan of ECO(5, 20, 5) design is obtained following Steps (i)-(iv) for \(t = 5\).

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 3 | 0 | 2 | 4 | 3 | 1 | 0 | 4 | 3 | 1 | 2 | 0 | 3 | 1 |
| 1 | 2 | 3 | 0 | 4 | 2 | 4 | 1 | 0 | 3 | 4 | 3 | 2 | 0 | 1 | 3 | 1 |
| 0 | 1 | 2 | 4 | 0 | 1 | 4 | 3 | 2 | 0 | 3 | 2 | 1 | 4 | 0 | 1 | 4 |
| 2 | 3 | 4 | 1 | 0 | 4 | 1 | 3 | 2 | 0 | 3 | 2 | 1 | 4 | 0 | 1 | 4 |
| 0 | 1 | 3 | 2 | 3 | 0 | 2 | 1 | 4 | 0 | 4 | 2 | 3 | 2 | 0 | 3 | 4 |
| 1 | 0 | 2 | 1 | 1 | 3 | 0 | 4 | 2 | 2 | 1 | 0 | 3 | 4 | 2 | 4 | 0 |
| 1 | 0 | 2 | 1 | 1 | 3 | 0 | 4 | 2 | 2 | 1 | 0 | 3 | 4 | 2 | 4 | 0 |

A plan of ECO(5, 20, \(p\)) design for \(2 \leq p \leq 5\) is obtained by considering any \(p\) rows from the above design. For example, the first three rows provide a plan of ECO(5, 20, 3) having ES 80%. A comparable COD(5, 15, 3) design with self first-order carryover effect that may not be desirable in some clinical trials.

Remarks

1. Every treatment in the last period is preceded by every other treatment in the first period only once and the rank(\(X\)) = (\(r + 1\))\(t + n + p - r - 2\), where \(X\) is coefficient matrix in model (1), \(X = \{R_0 : R_1 : R_2 \ldots \}, R_t : P : U\).

2. ECO(\(t\), \(t(t-1)\), \(t\) design, augmented by its own last row, is universally optimal for direct and first-order carryover effects under correlated observations (Martin and Eccleston 1998).

3. Estimation of Carryover Model for ECO(\(t\), \(t(t-1)\), \(p\)) Design

From the least squares analysis of model in (1) and following Martin and Eccleston (1998), we obtain the set of \(r + 1\)\((r = 0, 1, \ldots, p - 2\) reduced normal equations, one for direct treatment effects and remaining \(r\) for carryover effects, as

\[
C_{R0} \hat{\gamma}_0 = Q_{R0}, \tag{7}
\]

and

\[
C_{Re} \hat{\gamma}_e = Q_{Re}, e = 1, 2, \ldots, r, \tag{8}
\]

where

\[C_{R0} = C_{00} - \sum_{e=1}^{r} C_{0e} C_{ee} C_{00},\]

\[C_{Re} = C_{ee} - \sum_{f=0}^{r} C_{ef} C_{ff} C_{fe}; e = 1, 2, \ldots, r,\]

\[Q_{Re} = R'_e M_{Re} Y_{Re},\]

\[M_{Re} = I_n \otimes (I_{p-e} - F_{p-e}); F_p = \frac{J_p}{p},\]

\[Y_{Re} = Y_{ku}; k = e + 1, e + 2, \ldots, p; u = 1, 2, \ldots, n,\]

\[C_{ee} = D(e) - \frac{1}{n} N_e(e) N_p(e) - \frac{1}{p} N_u(e) N_p(e) + \frac{1}{np} N_e(e) N_u(e),\]

\[C_{ef} = C_{fe} = H(e) - \frac{1}{n} N_e(e) N_p(f) - \frac{1}{p} N_u(e) N_p(f) + \frac{1}{np} N_e(e) N_u(f),\]

\[D(e) = \text{diag}(r_1(e), \ldots, r_t(e)); r_i(e) = \sum_u n_{iu(e)}; i = 1, 2, \ldots, t,\]

\[H(e) = (h_{ij}^{(e)}) = \text{Concurrence of } e\text{th order carryover of } i\text{th treatment with } f\text{th order carryover of } j\text{th treatment}; i \neq j = 1, 2, \ldots, t\]

\[N_p(e) = \text{eth order carryover of } \times \text{period incidence matrix } = (i^{(e)}),\]

\[N_u(e) = \text{eth order carryover of } \times \text{unit incidence matrix } = (u_{i(e)}),\]

\[A^* = \text{generalized inverse of } A\text{ and everywhere above } e \neq f = 0, 1, \ldots, r.\]

Following Kiefer and Wynn (1981), the variance-covariance matrix for direct treatment effects vector \(\gamma_0\) and eth carryover effects vector \(\gamma_e\) are given by

\[
\begin{align*}
\text{var} (\hat{\gamma}_0) &= C_{R0}^{-1} \text{var} (Q_{R0}) C_{R0}^{-1}, \tag{9} \\
\text{var} (\hat{\gamma}_e) &= C_{Re}^{-1} \text{var} (Q_{Re}) C_{Re}^{-1}, \tag{10}
\end{align*}
\]

3.1 Estimation of Treatment Effects for ECO(\(t\), \(t(t-1)\), \(p(\leq t)\)) Design

For ECO(\(t\), \(t(t-1)\), \(p(\leq t)\)) design under the model (1),

\[
C_{ee} = \frac{(p - e)(t(p - 1) - e)}{p} (I_t - F_t), e = 0, 1, \ldots, r,
\]

and

\[
C_{ef} = \frac{(p - f)(t + e)}{p} (I_t - F_t), e < f = 0, 1, \ldots, r.
\]

Therefore,

\[
C_{R0} = K_0 (I_t - F_t),
\]

\[
K_0 = t(p - 1) - \frac{t^2}{p} \sum_{e=1}^{r} \frac{p - e}{t(p - 1) - e}, \tag{11}
\]

Statistics in Biopharmaceutical Research: May 2015, Vol. 7, No. 2

98
and

\[
C_{Re} = K_e (I_t - F_t),
\]

\[
K_e = \frac{p}{(p - e)^2} \sum_{f=0}^{e-1} \frac{(t + f)^2}{(p - f) (t(p - 1) - f)}
\]

\[
\left(\frac{p}{(p - e)^2} \sum_{f=0}^{e-1} \frac{(t + f)^2}{(p - f) (t(p - 1) - f)}\right) - \frac{(p - e)^2 e^{-1}}{p} \sum_{f=0}^{e-1} \frac{(t + f)^2}{(p - f) (t(p - 1) - f)},
\]

\[e = 1, 2, \ldots, r.\] (12)

So that,

\[
\hat{\gamma}_0 = C_{R0} Q_{R0}, \quad C_{R0} = K_0^{-1} I_t,
\] (13)

and

\[
\hat{\gamma}_e = C_{Re} Q_{Re}, \quad e = 1, 2, \ldots, r, \quad C_{Re} = K_e^{-1} I_t.\] (14)

### 3.2 Variance Balance of ECO(t, t(t - 1), p (≤ t)) Design

**Lemma 3.1.** For an ECO(t, n, p) design under model

\[
y = R_0 y_0 + R_1 y_1 + P \alpha + U \beta + \xi,
\]

\[
C_{R0} = K_0 (I_t - F_t), \quad K_0 = \frac{(t - 1)(t^3 + 2 t^2 - t - 1)}{t(t + 1)},
\]

\[
C_{R1} = K_1 (I_t - F_t), \quad K_1 = \frac{(t - 1)(t^3 + 2 t^2 - t - 1)}{t(t + 2)}.
\]

So from Equations (13) and (14) the estimate of direct treatment effects and first-order carryover effects are

\[
\hat{\gamma}_0 = (1/K_0)Q_{R0} \quad \text{and} \quad \hat{\gamma}_1 = (1/K_1)Q_{R1}.
\]

The variance expressions for any contrast of direct treatment effects and first-order carryover effects are as under:

\[
\text{var}(\gamma^c) = \left(\sum_{i=1}^{p-e-1} \rho_i R_i \otimes \left(\sum_{i=1}^{p-e-1} \rho_i Z_{(p-e)\alpha} M_{Re} R_i^* (I_t - F_t)^{-1}\right)\right)\xi^c \xi.
\]

\[
\text{var}(\gamma^c) = \left(\sum_{i=1}^{p-e-1} \rho_i R_i \otimes \left(\sum_{i=1}^{p-e-1} \rho_i Z_{(p-e)\alpha} M_{Re} R_i^* (I_t - F_t)^{-1}\right)\right)\xi^c \xi.
\]

where \(\gamma^c\) denotes a contrast.

2. For the ECO(t, t(t - 1), t) design, variance of the orthogonal direct treatment contrast through our approach is quite less as compared to that by factorial approach of Bose and Mukherjee (2000, 2003) for all possible values of correlation. For example in case of ECO(3, 6,
Table 1. Relative efficiencies of OLS approach estimates to factorial approach estimates

| Design      | Bose and Mukherjee (2000) | Bose and Mukherjee (2003) |
|-------------|---------------------------|---------------------------|
|             | 0  | 0.5 | 0.9       | 0  | 0.5 | 0.9       |
| ECO(3, 6, 3)| 240| 411 | 1846      | 230| 233 | 235       |
| ECO(4,12,4)| 1490| 2307| 9402      | 1363| 1396| 1417      |
| ECO(5,20,5)| 19197| 27672| 103438   | 15998| 16985| 17531     |

3), variance by our approach is 0.2604 and by Bose and Mukherjee (2000) approach is 0.625 when \( \rho = 0 \), that is, variance by Bose and Mukherjee (2000) approach is 240% of our approach. This means that variance of the orthogonal direct treatment contrast by our approach is 140% less as compared to Bose and Mukherjee (2000).

Table 1 shows relative efficiencies of above estimates by our approach as compared to those by factorial approach of Bose and Mukherjee (2000, 2003) considering equivalent variance-covariance structures as \( \sigma^2 V = \sigma^2 I_n \otimes [I_p + \rho \sum_{t=1}^{p-1} Z_{pt}] \) and \( \sigma^2 V = \sigma^2 I_n \otimes [I_p + \rho J_p] \), respectively. It is observed that relative efficiency increases with increasing \( \rho \) and \( t \). Although efficiencies are shown only for \( t = 3, 4, 5 \) but the same holds for \( t > 5 \).

3.3 Estimation of Treatment Effects for ECO\((t, t(t - 1), p(\leq t)) \) Design With Scattered Missing Observations

Unavoidable circumstances or accidents during implementation of the design may result in missing observations. Three cases of missing observations in ECO\((t, t(t - 1), p(\leq t)) \) are considered: (i) treatments were given to the units but observations were not recorded due to some reason, (ii) the experimenter was not in a position to give treatment to units because units could not appear at the time of scheduled period, and (iii) one or two units drop out from the beginning or choose to drop out intermittently.

In the first case, the complete design is executed. In the second case, all the carryover effects of the missing treatment is absent in the subsequent periods and raises complications in estimation. However, this situation can be handled by continuing with the planned sequence from the next period until the execution of the whole sequence. This may increase the length of the trial but the carryover structure remains simple. In the third case, unit dropout at beginning or immediately during the trial is handled by removing the whole treatment sequence from the design.

In the first and second cases, the matrices \( R_c, Y_c, \) and in the third case, all the matrices given in Equations (7)–(10) are written down as affected by the missing observations. Consider \( C_{ce}, C_{ef}, \) and \( M_{Re} \) in general form as

\[
C_{ce} = D^{(e)} - N_p^{(e)} (P'P)^{-1} N_p^{(ef)} - N_u^{(e)} (U'U)^{-1} N_u^{(ef)} + \frac{1}{np} N_u^{(e)} J N_u^{(ef)},
\]

Then estimation with its variance is carried out by solving Equations (7)–(10); however, the treatment effect estimates and hence the design loses the variance balanced property (4).

3.4 Estimation of Treatment Effects for ECO\((t, t(t - 1), p(\leq t)) \) Design at Interim Stage for Early Stopping

Both cost and successful execution of trial are important for any clinical investigation. Interim analysis is planned instead of fixed sample analysis to take advantage of the opportunities of early stopping. In terms of COD estimation, it is equivalent to having them robust to loss of late or last period/s, so that the experimenter can stop the design execution before the \( pt \)th period whenever executed design data show evidence for conclusion. The equineighbored nature (2) and (3) of the design ECO\((t, t(t - 1), p(\leq t)) \) shows that the design with \( p' \geq 2 \) is an ECO\((t, t(t - 1), p') \) design for which Theorem 3.2 exists. It follows that the variance balanced interim stage estimates of direct and carryover effects are given by Equations (7) and (8). This also includes as byproduct the case of several unit dropouts in the last period.

4. Numerical Illustration

The data in Table 2 are used to illustrate the computations involved in estimation of direct effects, first- and second-order carryover effects of treatments, and their
The roman figures I–XII denote 12 units, i–iv denote periods, and 1–4 numbers denote treatments.

4.1 Estimation With Complete Data

The key matrices terms in Equations (7) and (8) as per ECO(4,12,4) plan and data are \( n = 12, p = 4, Y_{R0} = \{15\ 15 \ 20 \ 15; 15 \ 15 \ 15 \ 15; 15 \ 15 \ 15 \ 20; 15 \ 15 \ 15 \ 15\} \) and the incidence matrices in Equation (7), direct effect of treatments 1–4 are obtained as 0.53, 0.53, 0.53, −1.59 and −1.28, −1.28, −1.28, 3.84. The variance of the treatment contrast \( \gamma_{ej} - \gamma_{ei}; i \neq j \) for \( e = 0, 1, 2, 3, 4 \) for \( Y_{R1} = [20 \ 15 \ 15 \ 15]: \) is 0.0391\( \sigma^2 \), 0.0546\( \sigma^2 \), and 0.0524\( \sigma^2 \), respectively, when \( \rho_s; s = 0, 1, 2, 3 \) is, respectively, 1, 0.9, 0.8, 0.7.

4.2 Estimation With One Missing Observation

Suppose the bold observation 15 shown in Table 2 is missing. Then, following case (1) of Section 3.3, using modified \( R^* = M_{R0}, Y_{R0} = [15 \ 20 \ 15: 15 \ 15 \ 15; \ldots \ : 15 \ 15 \ 15 \ 20], Y_{R1} = [20 \ 15: 15 \ 15; \ldots \ : 15 \ 15 \ 20], \) and the incidence matrices in Equation (7), direct effect of treatments 1–4 are obtained as 0.21, 0.37, 0.21, −0.79, respectively. Using Equation (8), first- and second-order carryover effect of the treatments 1–4 is obtained as 0.53, 0.40, 0.40, −1.33 and −1.28, −1.28, −1.28, 3.84, respectively. The variance of the treatments are obtained by Equations (9) and (10) but are not balanced.

4.3 Estimation With Unit Dropout

Suppose the first unit in Table 2 is a dropout at the beginning. Then, following case (3) of Section 3.3, we modify the incidence matrices in (7) and (8) as

| Period | I   | II  | III  | IV  | V   | VI  | VII | VIII | IX  | X   | XI  | XII |
|--------|-----|-----|------|-----|-----|-----|-----|-------|-----|-----|-----|-----|
| i      | 4(15)| 1(15)| 2(15)| 3(15)| 4(15)| 1(15)| 2(15)| 3(15) | 4(15)| 1(15)| 2(15)| 3(15)|
| ii     | 2(15)| 3(15)| 4(15)| 1(15)| 1(15)| 4(15)| 3(15)| 2(15) | 3(15)| 2(15)| 1(15)| 4(15)|
| iii    | 3(20)| 2(15)| 1(15)| 4(15)| 2(20)| 3(15)| 4(15)| 1(15) | 1(20)| 4(15)| 3(15)| 2(15)|
| iv     | 1(15)| 4(15)| 3(20)| 2(15)| 3(15)| 2(20)| 1(15)| 4(15) | 2(15)| 3(15)| 4(15)| 1(20)|

Table 2. Manipulated data from ECO(4,12,4)
variance balance and hence optimality in presence of carryover effects, the need for a large number of periods, that is, lengthy treatment sequence to be given to a unit for optimality under correlated errors, and loss of hard achieved properties when missing observations occur. The COD chosen for estimation in current article embeds within smaller COD with same number of treatments, and this property makes it special and useful in all above mentioned practical situations. For these designs, not only estimation of higher-order carryover effect is possible with variance balance but also the interim estimation of treatment effects and estimation under missing of three types as narrated in Section 3. The correlation structure used in estimation is that correlation between two observations from a unit at distance $s$ is $\rho_s; s = 0, 1, 2, \ldots, p - 1$. It is not as general as that in Martin and Eccleston (1998) and also not unrealistic as in Bose and Mukherjee (2003) who assumed it to be common at all distances.

The treatment effect estimates are interpreted relative to one another. For example, in the above illustration, direct effect estimates indicate that treatments 1, 2, and 3 make equal direct effect, which is higher than the direct effect of 4th treatment. Similarly, carryover effect estimates are interpreted. Alternatively, most researchers consider treatment contrasts instead. Using our methodology, estimates of treatment contrasts $\gamma_{e4} - \gamma_{e2}, \gamma_{2} - 3, 4$ for $e = 0, 1, 2, 3$ are, respectively, $-1, -2.12$, and $5.12$. This clearly shows that second-order carryover effect of treatment 4 is significant. To assess performance of our methodology, we carried out computations using easily executable methodologies. OLS estimation by Martin and Eccleston (1998) methodology for the above illustration resulted in direct treatment effect of contrast $\gamma_{e4} - \gamma_{e2}$ as $-1.33$ and first-order carryover effect of treatment contrast $\gamma_{t4} - \gamma_{t2}$ for $i = 1, 2, 3$ as $0, -0.1667$, and $0.1667$, respectively. It is somewhat contradicting with the design data. Next, computing estimates of direct, first-, and second-order carryover using PROC GLM in SAS resulted in values of each treatment contrasts $\gamma_{e4} - \gamma_{e2}$ for $i = 1, 2, 3$ as zeroes. This is clearly misleading. The treatment contrasts values indicate that performances of both existing methodologies are inadequate in presence of higher-order carryover.

Therefore, it is better to consider the model having higher-order carryover effect when the experimenter is not 100% sure about presence of the carryover effects. In most of the cases, these possibilities arise because either the experimenter does not know the proper washout period in advance or it is not feasible to set the lengthy washout period. In either case, it is better to consider higher-order carryover model on primary ground and analyze the data accordingly. If all higher-order carryover effects are absent, then the ex-

\begin{equation}
D^{(2)} = \begin{pmatrix}
6000 \\
0500 \\
0060 \\
0005 \\
\end{pmatrix},
H^{(12)} = \begin{pmatrix}
0222 \\
2021 \\
2102 \\
2220 \\
\end{pmatrix},
N_p^{(2)} = \begin{pmatrix}
0033 \\
0032 \\
0033 \\
0023 \\
\end{pmatrix},
N_u^{(2)} = \begin{pmatrix}
010111001110 \\
010010110110 \\
010100011001 \\
001101001001 \\
\end{pmatrix}.
\end{equation}

With modified incidence matrices, using $Y_{R0} = [15 15 15: 15 15 15 20: \ldots : 15 15 15 20]$ in Equation (7) gives direct effect of treatments 1–4 as 0.45, 0.42, −0.16, −0.71, respectively; using $Y_{R1} = [15 15 15 15 15 15: 15 15 20: \ldots : 15 20]$ and $Y_{R2} = [15 15: 15 15 15 15: \ldots : 15 20]$ in Equation (8) gives first- and second-order carryover of treatments 1–4 as 0.56, 1.26, 4.32, respectively. The variance of the treatments are obtained by Equations (9) and (10) but are not balanced.

### 4.4 Interim Stage Estimation

Suppose the experimenter had planned interim analysis at the end of third period, and hence wants to estimate the treatment effects after the execution of third period. Therefore, estimation of $\text{ECO}(4, 12, 3)$ embedded in Table 2 is carried out using Equations (7) and (8) with observation vectors $Y_{R0} = [15 15 20: 15 15 15: \ldots : 15 15 15]$, $Y_{R1} = [15 20: 15 15: \ldots : 15 15]$ and $p' = 3$ as explained in Section 3.4. In this case, the direct effects of treatments 1–4 are 0.26, 0.26, 0.26, −0.78 and their first-order carryover effects are 0.66, 0.66, 0.66, −1.98, respectively. The variance of the treatment contrast $\gamma_{cj} - \gamma_{cl}; i \neq j = 1, 2, 3, 4$ for $e = 0, 1$ by Equation (16) is $0.0509\sigma^2$ and $0.0561\sigma^2$, respectively, when $\rho_s; s = 0, 1, 2$ is, respectively, 1, 0.9, 0.8.

### 5. Discussion

A great deal of work has been done in the area of multi-period CODs including construction of plans and analysis under various models. Since multi-period designs cost more, they should be either versatile or the best. The COD$(t, t(t - 1), p)$ for specific values of $p$ is known to be optimal for direct effect of treatments, but not for its versatility. In practice, there are several hazards/hurdles in successful implementation of a multi-period COD. A design that can fill the gap between theoretical procedure and actual implementation gains popularity in practice. The main hurdles in application of COD are correlation among observations from same unit resulting in violation of OLS assumption, estimation of higher-order carryover effects at least for the purpose of confirmation, loss of
Estimation of Treatment and Carryover Effects in Optimal Cross-Over Designs for Clinical Trials

The experimenter moves to the traditional first-order carryover model to get the advantage of the precision because the design given in this article is optimal under traditional model.

APPENDIX: PROOF OF LEMMA 3.1 AND MATLAB CODE

Proof of Lemma 3.1.

\[ \text{var}(Q) = \text{var}(R^* M R Y R) \]
\[ = R^* M R \left( \sigma^2 I + \sum_{i=0}^{p-1} \rho_i Z_i (p-c)^t \right) R^* \]
\[ = \sigma^2 R^* \left( I + \sum_{i=0}^{p-1} \rho_i Z_i (p-c)^t \right) R^* \]
\[ = \sigma^2 \sum_{i=0}^{p-1} \rho_i R^* \left( I (p-c) - F(p-c) \right) Z_i (p-c) (p-c)^t R^* \]

Matlab Code

The code given below estimates the direct and carryover for ECO\((t, t(t-1), p (\leq t))\) and ECO\((t, t(t-1), p^t)\) design. The code for scattered missing observations and unit dropout in ECO design is available through E-mail on request to the author. Experimenter has to set the design parameters as shown between star boarders.

```matlab
function star = fullD(p, k)
    incr = 0;
    for inci = 1:n
        for incr = incr + 1;
            if incj = (p-1)
                Rot(incr,Design(inci,inci),inci) = 1;
            else
                if (incj-inci) > 0
                    Rot(incr,Design(inci,inci),inci,inci) = 1;
                end
            end
        end
    end

    Design = fullD(1:p,)
    incr = 0;
    for incj = 1:p
        incr = incr + 1;
        if incj = (p-1)
            Rot(incr,Design(inci,inci),inci) = 1;
        end
    end
    end

    P = kron(ones(n,1),eye(p));
    U = kron(eye(n),ones(p,1));
    srno = 0;
    for i = 1:p-1
        Dm(:,i) = Rot(:,i)^Ratm(1:n,p);
        end
    end
    for j = 1:p-1
        Ntp(:,i) = Rot(:,i)^P;
        end
    end
    for i = 1:p-1
        Ntu(:,i) = Rot(:,i)^U;
        end
    end
    for j = 1:p-1
        H(:,i,j) = Rot(:,i)^Rot(:,j);
        end
    end
    end
    for i = 1:p-1
        for j = 1:p-1
            C(:,i,j) = H(:,i,j)^-1
            (1/n)Ntp(:,i)Ntp(:,j)^-1
            (1/p)Nmu(:,i)Nmu(:,j)^+1
            (1/n)pNmu(:,i)ones(m)Nmu(:,j);
        end
    end
    end
    end
    for i = 1:p-1
        for j = 1:p-1
            C(:,i,j) = H(:,i,j)^-1
            (1/n)Ntp(:,i)Ntp(:,j)^-1
            (1/p)Nmu(:,i)Nmu(:,j)^+1
            (1/n)pNmu(:,i)ones(m)Nmu(:,j);
        end
    end
    end
    end
    end
    end
```

103
for i = 1:p-1
    if i == (p-1)
        MR(:,:,p-1)=kron(eye(n),eye(p-ones(p)/(1/p)));
    else
        MR(:,:,p-1)=kron(eye(n),eye(p-ones(p-1)/(1/p-1)));
    end
end

for i = 1:p-1
    if i == (p-1)
        QR(:,:,p-1)=QR(:,:,p-1)*MR(:,:,p-1);
    else
        QR(:,:,i)=Rstar(1:(n-1:p-1),:,:,i)*MR(1:(n-1:p-1),:,:,i)*YR(1:(n-1:p-1),:,:,i);
    end
end

dvarcov = pinv(CR(:,:,p-1))'*Rot(:,:,p-1)'*MR(:,:,p-1)*kron(eye(n),delta(1:p,1:p))'*MR(:,:,p-1)*Rot(:,:,p-1)'*pinv(CR(:,:,p-1));

end

VarDirect = ldamda'dvarcov'ldamda;
for i = 1:p-2
    cvarcov = pinv(CR(:,:,i))'*Rstar(1:(n-1:p-1),:,:,i)*MR(1:(n-1:p-1),:,:,i)*kron(eye(n),delta(1:p-1:i,:,:,i))'*MR(1:(n-1:p-1),:,:,i)*YR(1:(n-1:p-1),:,:,i); *pinv(CR(:,:,i));
end

EffectCarry(:,:,i) = ldamda'cvarcov'ldamda;
end

EffectDirect = Effect(:,:,p-1)
VarDirect
for i = 1:p-2
    EffectCarry(:,:,i) = Effect(:,:,i);
end

if p>2
    EffectCarry
    VarCarry
end

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