Likelihood based missing data analysis in multivariate crossover trials

Savita Pareek¹, Kalyan Das¹, and Siuli Mukhopadhyay¹, ²

¹Department of Mathematics, Indian Institute of Technology Bombay, Mumbai 400 076, India

Abstract

For gene expression data measured in a crossover trial, a multivariate mixed effects model seems to be most appropriate. Standard statistical inference fails to provide reliable results when some responses are missing. Particularly for crossover studies, missingness is a serious concern as the trial requires a small number of participants. A Monte Carlo EM (MCEM) based technique has been adopted to deal with this situation. Along with estimation, a MCEM likelihood ratio test (LRTs) is developed for testing the fixed effects in such a multivariate crossover model with missing data. Intensive simulation studies have been carried out prior to the analysis of the gene expression data.

Keywords: Multivariate crossover trials; Monte Carlo EM algorithm; MCEM LRTs;

1 Introduction

In many clinical studies, we may come across crossover trials with two or more response variates with subject dropouts and incomplete data. For example, one may consider the measurement of both systolic (SBP) and diastolic (DBP) blood pressure in each period (Grender and Johnson⁶) or recording blood sugar levels at multiple time points in each period (Putt and Chinchilli¹⁴) or microarray gene expression profiles of subjects measured in each period (Leaker et al.¹¹) with incomplete data. Using conventional univariate crossover models based on only complete cases to analyse such missing data
and multivariate data, may lead to incorrect and biased parameter and variance estimates. In a crossover trial, usually a smaller number of participants as compared to cross-sectional studies are recruited as the same subjects are switched between all treatment groups. Thus, ignoring subject dropout information and performing a complete case analysis may lead to highly underpowered studies and biased estimates. Similarly, ignoring the correlated nature of the multiple responses recorded in each period and fitting separate crossover models to each response may lead us to inaccurate conclusions about the intra and inter subject variabilities. Thus, to address the issue of missing responses in a multivariate crossover setup, we propose a mixed effect model approach and an Monte Carlo EM (MCEM) based estimation method.

For a detailed review of univariate crossover designs, refer to the books by (Senn[17]; Jones and Kenward[9]). In comparison, crossover trials with multiple responses measured in each period have been addressed by fewer researchers, namely (Grender and Johnson[6]; Chinchilli and Esinhart[2]; Putt and Chinchilli[14]; Tudor et al.[19]). To tackle the presence of missing/incomplete data specifically for the AB/BA design under the MAR or MNAR missing mechanism one may refer to (Patel[13]; Ho et al.[7]; Matthews and Henderson[12]). For more general univariate crossover designs with incomplete data references are namely, (Richardson and Flack[15]; Chow and Shao[3]; Basu and Santra[1]; Rosenkrantz[16]). However, none of the works cited deal with missingness in a multivariate crossover design setup.

In this article we model and analyse multivariate crossover studies in the presence of missing at random outcomes. This work is motivated by a $3 \times 3$ crossover trial studying the effect of two single doses of oral prednisone (10 and 25 mg) with placebo on biomarkers of mucosal inflammation and transcriptomics (Leaker et al.[11]). The gene expression changes of subjects were measured in each period of the trial giving rise to multiple responses in a crossover framework. A total of seventeen subjects assigned to three treatment sequences were enrolled in the crossover study, however some missing observations (particularly) in the third period were observed. Our task here is to fit an appropriate statistical model to the crossover trial with microarray expression data taking into account the multivariate structure of the data and the missing responses. A Monte Carlo EM based mixed model analysis is used to fit and estimate the treatment and gene effects along with the intra and inter subject variability in the presence of missing at random outcomes. The mixed model assumed, includes the fixed effects of treatment,
period and genes, and their interactions while the random effects are subject and subject gene interaction effect. We used maximum likelihood estimation coupled with the MCEM algorithm to determine the estimated model parameters and variance components. For finding the asymptotic covariance matrix, multiple imputation method of (Els and Ryan[5]) was considered. Likelihood ratio tests (LRTs) based on the MCEM estimators were formulated. We used detailed simulations to study the properties of the estimators and power of the LRTs proposed. A real data example based on the gene expression levels measured in a $3 \times 3$ crossover study is used to illustrate the statistical model and its estimation.

To the best of our knowledge this is the first work involving multivariate crossover studies with missing responses. Though originally developed for agricultural sciences, crossover trials are nowadays frequently used in clinical trials and biological studies to evaluate treatment effects. These trials are most useful for comparing treatments for chronic or long term diseases, such as asthma, rheumatism, hypertension etc. Note that one useful advantage of crossover studies is that they require a smaller number of participants. However, due to smaller number of recruits, reliable statistical inference is harder to make when some responses are randomly missing.

2 Case study: multivariate crossover trial of oral prednisone

We use a gene expression dataset from (Leaker et al.[11]) as a case study. The dataset is publicly available from the NCBI Gene Expression Omnibus (Clough and Barrett[4]). In the gene expression study results from a randomized double-blind, placebo-controlled, three-period, crossover trial are considered to evaluate the effects of two single doses of oral prednisone (10 mg, 25 mg) on inflammatory mediators measured in nasal exudates after nasal allergen challenge in susceptible individuals with allergic rhinitis. All subjects have a history of seasonal asthma rhinitis to grass pollen and also a positive result from the intraepidermal skin prick test to grass pollen extract. There are a total of seventeen subjects enrolled in the study, assigned to three treatment sequences/groups. Some missing observations (particularly) in the third period are also recorded. The main interest here is to study the effect of treatments and genes on allergic reaction to grass pollen.
The crossover trial consists of 3 distinct sequences of treatments assigned to a total of 17 subjects. The sequences of treatments are: Sequence 1 (10 mg, placebo, 25 mg), Sequence 2 (25 mg, 10 mg, placebo), Sequence 3 (placebo, 25 mg, 10 mg). Subject 1 to subject 6 are in sequence 1, subject 7 to subject 12 are in sequence 2 and subject 13 to subject 17 are in sequence 3. For each subject, 10 gene expressions are measured by giving treatments (10 mg prednisone, 25 mg prednisone and placebo) in three periods with a washout period of 4 weeks. As an example, Sequence 1 indicates that 10 mg prednisone is given to the subjects (subject 1 to subject 6) at period 1 followed by a washout, period 2 placebo is given and again after a washout, 25 mg prednisone is given at period 3. The study comprises of missing responses in period 3 of various sequences. In sequence 1, response values of subjects 2, 5 are missing; in sequence 2, response values of subjects 8, 10 are missing and in sequence 3, response values from subject 14 are missing. The overall percentage of missing responses in the data is 9%, with 11% missing in sequences 1 and 2, and 6% in sequence 3.

Prior to model fitting we ran some exploratory analysis of the gene data using complete cases. The following facts were revealed:

(i) Gene by treatment and gene by period interactions were seen to be almost absent for each treatment sequence, see fig. 1.

(ii) From fig. 2 it is fair to assume no subject by gene interaction effect exists in our model.

Considering these two points, in the following section, we frame a specific model that pertains to the above microarray expression data.

3 Model and notation

Suppose \( y_{ijkl} \) denotes measurement of the \( l \)th gene expression from the \( k \)th subject in the \( j \)th period assigned to the \( i \)th treatment sequence, where \( i = 1, 2, \ldots, s \); \( j = 1, 2, \ldots, p \); \( k = 1, 2, \ldots, n_i \) and \( l = 1, 2, \ldots, m \). So there are in total \( n \) subjects, where \( n = \sum_{i=1}^{s} n_i \). Assuming a normal random effects model we may write,

\[
y_{ijkl} = \mu + \pi_j + \tau_{d[i,j]} + g_l + s_{ik} + e_{ijkl} \tag{1}
\]

where \( \mu \) is an intercept, \( \pi_j \) is the fixed effect of the \( j \)th period, \( \tau_{d[i,j]} \), \( (d[i,j] = 1, 2, \ldots, t) \) is the fixed effect associated with the treatment applied in period
Figure 1: Period by gene and treatment by gene interaction plots; Plots on the left (A, B, C) is period by gene interaction plots for sequences 1, 2 and 3 respectively. Plots on the right (D, E, F) is treatment by gene interaction plots for sequences 1, 2 and 3 respectively.
Figure 2: Subject by gene interaction plot; Plots A, B, C is subject by gene interaction plots for sequence 1, 2 and 3 respectively.
of the sequence \(i\), \(g_i\) is the fixed effect of the \(l\)th gene, \(s_{ik}\) is a random effect associated with the \(k\)th subject in sequence \(i\), and \(e_{ijkl}\) is a random error. We assume that, \(e_{ijkl} \sim \text{ind } N(0, \sigma_e^2)\) \(\forall i, j, k, l\), and \(s_{ik} \sim \text{ind } N(0, \sigma_s^2)\) \(\forall i, k\). Also, \(s_{ik}, e_{ijkl}\) are independent. Note, we have not considered an interaction effect of gene with period and treatment in the above model, in accordance with the exploratory analysis results for the case study data. Based on the above assumptions, we have \(\text{var}(y_{ijkl}) = \sigma_e^2 + \sigma_s^2\), and \(\text{cov}(y_{ijkl}, y_{ij'l'}) = \sigma_s^2\) for all \(j \neq j', l \neq l'\). Thus, variance-covariance matrix corresponding to the \(k\)th subject administered the \(t\)th treatment sequence is given by,

\[
\text{var}(y_{ik}) = (\Sigma_{ik})_{pm \times pm} = \\
\begin{bmatrix}
\sigma_e^2 R & \sigma_s^2 1_m 1_m^T & \ldots & \sigma_s^2 1_m 1_m^T \\
\sigma_s^2 1_m 1_m^T & \sigma_e^2 R & \ldots & \sigma_s^2 1_m 1_m^T \\
\vdots & \vdots & \ddots & \vdots \\
\sigma_s^2 1_m 1_m^T & \sigma_s^2 1_m 1_m^T & \ldots & \sigma_e^2 R \\
\end{bmatrix}
\]

where, \(R = I_m + \frac{\sigma_s^2}{\sigma_e^2} 1_m 1_m^T\), and \(1_m^T = (1, 1, \ldots, 1)_{1 \times m}\).

Using matrix notations we rewrite equation 1 as

\[
y_i = X_i \beta + Z_i b_i + e_i, \quad i = 1, 2, \ldots, s
\]  

(2)

where,

(i) \(y_i = (y_{i1}^T, y_{i2}^T, \ldots, y_{iq}^T, \ldots, y_{ipqm})^T\) is a response vector of length \(pmn_i\), and

\(y_{iq} = (y_{1iq1}, \ldots, y_{ipqm})^T\).

(ii) \(X_i = (X_{i1}^T, X_{i2}^T, \ldots, X_{iq}^T, \ldots, X_{ipqm})^T_{pmn_i \times (p+t+m-2)}\) is the design matrix corresponding to the fixed effects, and

\(X_{iq} = (1_p \otimes 1_m, ((1_{p-1} \otimes 1_m)^T, 0_{m \times (p-1)})^T_{pm \times (p-1)}, T_{pm \times (t-1)}, 1_p \otimes (I_{m-1}, 0_{m-1})^T)\).

The \(pm \times (t-1)\) matrix of treatment effects is represented as \(T = (T_1^T, \ldots, T_p^T)^T\), where \(T_u = (1_m \otimes (a_1, \ldots, a_{t-1}))_{m \times (t-1)}\) for \(u=1, \ldots, p\), and \(a_t\) is an indicator variable which takes value 1 if the \(l\)th treatment is assigned to the \(u\)th period and 0 otherwise.

(iii) \(Z_i = I_{n_i} \otimes 1_{pm}\) is the design matrix corresponding to the random effects.

(iv) Moreover, \(b_i = (s_{i1}, s_{i2}, \ldots, s_{in_i})^T\) is the \(n_i \times 1\) vector of random effects, and
\[ e_i = (e_{i1}, e_{i2}, \ldots, e_{in})^T \] is the \( pmn_i \times 1 \) random error vector, where \( e_{iq} = (e_{i1}, \ldots, e_{ipq})^T \), it is assumed
\[ e_i \sim N(0, \sigma^2 I_{pmn_i}); \quad b_i \sim N_n(0, D_i), \quad D_i = \sigma^2 I_n. \]

(v) The parameters of interest are,
\[ \beta = (\mu, \pi_1, \ldots, \pi_{p-1}, \tau_1, \ldots, \tau_{t-1}, g_1, \ldots, g_{m-1}) \] and \( \sigma^2 = (\sigma_e^2, \sigma_s^2) \).

Under the above assumptions, the conditional distribution of \( y_i \) given the random effects \( b_i \) is of the form
\[ y_i | \beta, \sigma^2_e, b_i \sim N_{pmn_i}(X_i \beta + Z_i b_i, \sigma^2_e I_{pmn_i}), \]
while the marginal distribution of \( y_i \) is
\[ y_i | \beta, \sigma^2_e, D_i \sim N_{pmn_i}(X_i \beta, \Sigma_i), \quad \Sigma_i = Z_i D_i Z_i^T + \sigma^2_e I_{pmn_i}. \]

4 Estimation with missing at random responses

In this section we illustrate how an EM type algorithm can be used for maximum likelihood estimation (MLE) in multivariate crossover model in a missing set up. We have used the MCEM algorithm of (Wei and Tanner\[20\]) for parameter estimation. Note that for ignorable missing (MAR) assumption in the data, it is not necessary to incorporate the parametric model for the missing data mechanism into the complete data log likelihood. Therefore the complete data log likelihood for the \( i \)-th sequence is given by \( f(y_i, b_i) \), where \( y_i \) may contain missing values. We assume the covariates to be fully observed.

In the E-step we calculate the expected value of the complete data log likelihood given the observed data and current parameter estimates. Suppose, \( y_i = (y_{mis,i}^T, y_{obs,i}^T)^T \) where \( y_{mis,i} \) is the \( m_i \times 1 \) vector of missing components of \( y_i \). Note, both \( b_i \) and \( y_{mis,i} \) are unobserved, and must be integrated over. To execute the EM algorithm the steps listed were followed from (Ibrahim and Molenberghs\[8\]). Thus the E-step for \( i \)-th sequence at \((t+1)\)th iteration.

\[
Q_i(\gamma | \gamma^{(t)}) = E\left(l(\gamma; y_i, b_i | y_{obs,i}, \gamma^{(t)})\right)
= \int \int \log \left[f(y_i | \beta, \sigma^2_e, b_i)\right] f(y_{mis,i}, b_i | y_{obs,i}, \gamma^{(t)}) \; db_i \; dy_{mis,i}
+ \int \int \log \left[f(b_i | D_i)\right] f(y_{mis,i}, b_i | y_{obs,i}, \gamma^{(t)}) \; db_i \; dy_{mis,i}
\equiv I_1 + I_2,
\]

(3)
Thus, \( \gamma^{(t)} = (\beta^{(t)}, \sigma_\epsilon^{2(t)}, \sigma_\epsilon^{2(t)}) \).

To integrate out \( b_i \) from \( I_1 \) and \( I_2 \) we write

\[
f(y_{mis,i}, b_i | y_{obs,i}, \gamma^{(t)}) = f(b_i | y_i, \gamma^{(t)}) f(y_{mis,i} | y_{obs,i}, \gamma^{(t)})
\]

where, \( b_i | y_i, \gamma^{(t)} \sim N(b_{0i}^{(t)}, \Sigma_{0i}^{(t)}) \) and,

\[
b_{0i}^{(t)} = D_i^{(t)} Z_i (Z_i D_i^{(t)} Z_i + \sigma_\epsilon^{2(t)} I_{pmni})^{-1} (y_i - X_i \beta^{(t)})
\]

\[
\Sigma_{0i}^{(t)} = D_i^{(t)} - D_i^{(t)} Z_i (Z_i D_i^{(t)} Z_i + \sigma_\epsilon^{2(t)} I_{pmni})^{-1} Z_i D_i^{(t)}
\]

\[
= \left[ \sigma_\epsilon^{-2(t)} Z_i \Sigma_i + (D_i^{(t)})^{-1} \right]^{-1}.
\]

Thus,

\[
I_1 = -\frac{pmni}{2} \log 2\pi - \frac{pmni}{2} \log \sigma_\epsilon^2 - \frac{1}{2\sigma_\epsilon^2} (Tr(Z_i^T Z_i \Sigma_{0i}^{(t)}))
\]

\[
+ \int (y_i - X_i \beta - Z_i b_{0i}^{(t)})^T (y_i - X_i \beta - Z_i b_{0i}^{(t)}) f(y_{mis,i} | y_{obs,i}, \gamma^{(t)}) dy_{mis,i}.
\]

(4)

and,

\[
I_2 = -\frac{(m+1)n_i}{2} \log 2\pi - \frac{1}{2} \log(\det D_i) - \frac{1}{2} Tr(D_i^{-1} \Sigma_{0i}^{(t)})
\]

\[
- \frac{1}{2} \int (b_{0i}^{(t)} D_i^{-1} b_{0i}^{(t)}) f(y_{mis,i} | y_{obs,i}, \gamma^{(t)}) dy_{mis,i}.
\]

(5)

We sample \( v_{i1}, v_{i2}, \ldots, v_{ic} \) from \([y_{mis,i} | y_{obs,i}, \gamma^{(t)}]\), which has the density of the form,

\[
y_{mis,i} | y_{obs,i}, \gamma^{(t)} \sim N_{mi} (\mu_1 + \Sigma_{12} \Sigma_{22}^{-1} (y_{obs,i} - \mu_2), \Sigma_{11} - \Sigma_{12} \Sigma_{22}^{-1} \Sigma_{21}).
\]

(6)

where,

\[
\begin{pmatrix}
  y_{mis,i} \\
  y_{obs,i}
\end{pmatrix}
= \begin{pmatrix}
  X_{mi \times 1}^{(1)} \\
  X_{li \times 1}^{(2)}
\end{pmatrix} \beta + \begin{pmatrix}
  Z_{mi \times 1}^{(1)} \\
  Z_{li \times 1}^{(2)}
\end{pmatrix} b_i + \begin{pmatrix}
  e_{mi} \\
  e_{li}
\end{pmatrix}; \quad m_i + l_i = pmni
\]

\[
y_i = \begin{pmatrix}
  y_{mis,i} \\
  y_{obs,i}
\end{pmatrix} \sim N(\mu, \Sigma),
\]

9
Obviously, the E-step for all the sequences is given by

\[
\mu = \left( \begin{array}{c}
\mu_1 \\
\mu_2 
\end{array} \right) = \left( \begin{array}{c}
X_i^{(1)} \beta \\
X_i^{(2)} \beta 
\end{array} \right),
\]

\[
\Sigma = \left( \begin{array}{cc}
\Sigma_{11} & \Sigma_{12} \\
\Sigma_{21} & \Sigma_{22}
\end{array} \right) = \left( \begin{array}{cc}
Z_i^{(1)} D_i Z_i^{(1)T} & Z_i^{(1)} D_i Z_i^{(2)T} \\
Z_i^{(2)} D_i Z_i^{(1)T} & Z_i^{(2)} D_i Z_i^{(2)T}
\end{array} \right) + \sigma_e^2 \left( \begin{array}{cc}
I_{n_i} & 0 \\
0 & I_{l_i}
\end{array} \right).
\]

Using the data \( y_i^{(k)} = (v_{ik}, y_{ob,i}^T) \) and \( b_{0i}^{(tk)} = \Sigma_{0i}^{(t)} Z_i^T (y_i^{(k)} - X_i \beta^{(t)}) \sigma_e^{(t)}, \) eq. (4), eq. (5) and eq. (6) the E-step for the \( t \)th sequence (eq. (3)) in the \((t + 1)\)th iteration takes the form

\[
Q_i(\gamma|\gamma^{(t)}) = -\frac{pmn}{2} \log 2\pi - \frac{pmn}{2} \log \sigma_e^2
- \frac{1}{2\sigma_e^2} \left( Tr(Z_i^T Z_i \Sigma_{0i}^{(t)}) + \frac{1}{c_i} \sum_{k=1}^{c_i} (y_i^{(k)} - X_i \beta^{(t)} - Z_i b_{0i}^{(tk)}) (y_i^{(k)} - X_i \beta^{(t)} - Z_i b_{0i}^{(tk)}) \right)
- \frac{1}{2} \left( m + 1 \right) n_i \log 2\pi - \frac{1}{2} \log(\det D_i) - \frac{1}{2} Tr(D_i^{-1} \Sigma_{0i}^{(t)})
- \frac{1}{2c_i} \sum_{k=1}^{c_i} b_{0i}^{(tk)} D_i^{-1} b_{0i}^{(tk)}.
\]

Obviously, the E-step for all the sequences is given by

\[
Q(\gamma|\gamma^{(t)}) = \sum_{i=1}^{s} Q_i(\gamma|\gamma^{(t)}).
\]

In the M-step, we maximize \( Q(\gamma|\gamma^{(t)}) \). The closed forms are obtained for \((\beta, \sigma_e^2, \sigma_s^2)\) by computing

\[
\frac{\partial Q}{\partial \beta} = 0, \quad \frac{\partial Q}{\partial \sigma_e^2} = 0, \quad \frac{\partial Q}{\partial \sigma_s^2} = 0
\]

\[
\beta^{(t+1)} = \left( \sum_{i=1}^{s} X_i^T X_i \right)^{-1} \sum_{i=1}^{s} X_i^T \frac{1}{c_i} \sum_{k=1}^{c_i} (y_i^{(k)} - Z_i b_{0i}^{(tk)})
\]

\[
\sigma_e^{2(t+1)} = \frac{1}{pmn} \sum_{i=1}^{s} \left( \frac{1}{c_i} \sum_{k=1}^{c_i} (y_i^{(k)} - X_i \beta^{(t+1)} - Z_i b_{0i}^{(tk)}) (y_i^{(k)} - X_i \beta^{(t+1)} - Z_i b_{0i}^{(tk)}) \right)
+ Tr(Z_i^T Z_i \Sigma_{0i}^{(t)})
\]

\[
\sigma_s^{2(t+1)} = \frac{1}{n} \sum_{i=1}^{s} \left( Tr(\Sigma_{0i}^{(t)}) + \frac{1}{c_i} \sum_{k=1}^{c_i} b_{0i}^{(tk)T} I_{n_i} b_{0i}^{(tk)} \right)
\]
The above $E$ and $M-$ steps are repeated until convergence is achieved.

To find the asymptotic covariance matrix, the multiple imputation technique has been considered. For imputing a missing value we sampled from $[b_i, y_{miss,i} | \hat{\gamma}, y_{obs,i}]$ following the steps below, note $\hat{\gamma} = (\hat{\beta}, \hat{\sigma}_e^2, \hat{\sigma}_s^2)$ are the MCEM estimates,

(i) Initial estimates $(b_{i0}, y_{miss,i0})$ were taken from the respective marginal distributions i.e. $b_i \sim N(0, \sigma_s^2 I_n)$ and $y_{miss,i} \sim N(X_i^{(1)} \beta, Z_i^{(1)} D_i Z_i^{(1)^T} + \sigma_e^2 I_{m_i})$.

(ii) At the $k(\geq 1)$th iteration $(b_{ik}, y_{miss,ik})$ were sampled using $b_{ik}$ from $b_i | y_{obs,i}, y_{miss,ik-1}$ and $y_{miss,ik}$ from $y_{miss,i} | y_{obs,i}, b_{ik}$.

We generated $b$ such samples of $[b_i,y_{miss,i}]$. The parameter estimates and their variances were computed based on the $b$ sets of imputed values. Repeating the procedure $m_0$ times the final variance estimates were taken to be: (mean of the imputed variances) $+ (1 + 1/m_0)$ (empirical variances of the imputed point estimates).

5 Hypothesis testing and Power computation

A MCEM-LRT test is developed for testing the fixed effects in a multivariate crossover model with missing data. Here our interest is in testing the fixed effects, however the LRT procedure may also be used to test the variance components. Specifically, for testing the treatment effect we frame our hypotheses as

$$H_0 : \tau_1 = \tau_2 = \ldots = \tau_t \text{ vs } H_1 : \tau_1, \tau_2, \ldots, \tau_t \text{ are not all equal}$$

The LRT statistic is $\Lambda = 2 \log L(\hat{\theta}_{full}) - 2 \log L(\hat{\theta}_{reduced})$, where $\log L(\hat{\theta}_{full})$ is the likelihood function for the full model containing all the parameters, and $\log L(\hat{\theta}_{reduced})$ is the likelihood function value for the reduced model under $H_0$. Under the normality assumption and certain other regularity conditions (Shao\cite{18}), when $H_0$ is true, $S^2$ follows a central $\chi^2_{df}$ where $df$ is the difference between the number of parameters in the full model and the reduced model. The null hypothesis, $H_0$, is rejected if the observed value of $S^2$ exceeds the $(1 - \alpha)^{th}$ quantile of $\chi^2_{df}$. 

11
For testing the period effect or the fixed effect of the response variates we may use the respective hypotheses,

\[ H_0 : \pi_1 = \pi_2 = \ldots = \pi_p \text{ vs } H_1 : \pi_1, \pi_2, \ldots, \pi_p \text{ are not all equal}, \]

\[ H_0 : g_1 = g_2 = \ldots = g_m \text{ vs } H_1 : g_1, g_2, \ldots, g_m \text{ are not all equal}. \]

Note for EM-LRT we have assumed that the central limit theorem holds for the MCEM estimator. Thus, in the next section we carry out extensive simulations to assess the EM-LRT test and its power for various combinations of sample size and % of missingness.

6 Simulation study

We discuss a detailed simulation study to assess the performance of the MCEM estimators and the LRT test based on these estimators. For data generation, we assumed a crossover trial with two treatment sequences \{AB, BA\} in two periods. In each period four response variates were measured. The model is represented as,

\[
y_{ijkl} = \beta_0 + \beta_{\text{per}\text{-Period}_1} + \beta_{\text{Trt}_1} + \beta_{\text{Res}_1} + \beta_{\text{Res}_2}
+ \beta_{\text{Res}_3} + s_{ik} + e_{ijkl}; \quad i, j = 1, 2, \quad l = 1, 2, 3, 4, \quad k = 1, 2, \ldots 50
\]

where \(y_{ijkl}\) denotes the \(l^{th}\) response value from the \(k^{th}\) subject in the \(j^{th}\) period of the \(i^{th}\) sequence; \(\beta_0\) is the intercept; \(\text{Period}_1\) is an indicator variable corresponding to the first period effect; \(\text{Trt}_1\) is an indicator variable corresponding to the treatment effect \(A\) and \(\text{Res}_1, \text{Res}_2, \text{Res}_3\) are indicator variables corresponding to the response variates, where \(\text{Res}_i = 1\) for the \(i^{th}\) response variate and 0 otherwise for \(i = 1, 2, 3\). Also, as defined before \(s_{ik}\) is the subject specific random effect and \(e_{ijkl}\) is the error term.

\[ s_{ik} \sim N \left( 0, \sigma_s^2 \right), \quad e_{ijkl} \sim N \left( 0, \sigma_e^2 \right); \quad s_{ik} \perp e_{ijkl} \]

In matrix notations,

\[ y_i = X_i \beta + Z_i b_i + e_i, \]

where, for \(i = 1, 2\), \(e_i \sim N_{400} (0, 1.44I), b_i \sim N_{50} (0, 0.49I), X_i \) and \(Z_i\) matrices are constructed similarly as in eq. (2). The true values of the components of \(\beta\) are given in the first column of Table 1.
We considered different probabilities of missingness (15%, 25%, 35%) in each sequence and the missing mechanism to be MAR. Since the non-response model is assumed to be ignorable (Laird\cite{10}) we use a Bernoulli random variable with probability 0.15, 0.25, 0.35, respectively to generate the missing data. The MCEM algorithm discussed in section 4 is used for parameter estimation and SEs (standard errors) of the estimators are obtained via the imputation method. At every iteration of the MCEM algorithm, one thousand samples were generated for missing observations.

The simulations were repeated 500 times. Table 1 shows the average simulation results in terms of parameter estimates, SEs, bias, and empirical coverages for different missing percentages. Absolute average bias was computed as $|\sum_{w=1}^{500}(\hat{\beta}_{uw} - \beta_u)|/500$, where $\hat{\beta}_{uw}$ is the $u$th component of $\hat{\beta}$ for the $w$th simulation and $\beta_u$ is the true value. Whereas, the empirical coverage probability (ECP) was taken to be the proportion of the times the 95% asymptotic confidence interval of $\hat{\beta}_{uw}$ contained the true parameter value $\beta_u$ in 500 simulations. To compute the SEs, one hundred imputations with a burn-in of 1000 were used.

From Table 1, one notes that absolute average biases for 15% missing responses, ranges between 2% - 5%. As we increase the % of missingness to 35%, the range of the average bias change to 4% - 11%. The average MSEs of the estimates range from 1% - 3% for all three missing percentages, showing that the corresponding standard errors are also quite low. These simulation results imply that the MCEM estimators work well for finite samples.

In order to check the asymptotic normality of the MCEM estimators, empirical probability density curve of each of the standardized estimator was compared with the standard normal density curve. Figure 3 displays empirical PDF of the standardized MCEM estimates with varying missing percentages and number of simulations. From the fig. 3, note as the number of simulations increase, the MCEM estimators (standardized one) are closer to asymptotic normality. The R programs are available on request from the first author.

Further, we tested the following hypothesis using the MCEM-LRT procedure detailed in section 5 and computed the power of the following tests:

(i) Tests for response variate effects: $H_0: \beta_{r_1} = \beta_{r_2} = \beta_{r_3} = 0$ versus $H_1$: At least one $\beta_{r_i} \neq 0$ for $i = 1, 2, 3$.

(ii) We also performed the pairwise tests of response variates,
Figure 3: Empirical PDF of standardized MCEM estimates; Plots (A, C) corresponds to cases with 100 simulated data sets when missingness is 15% and 25% respectively. Plots (B, D) corresponds to cases with 500 simulated data sets when missingness is 15% and 25% respectively.
Table 1: Simulation results based on five hundred samples

| Parameter | MLE | SE  | Bias | MSE | ECP |
|-----------|-----|-----|------|-----|-----|
| Intercept (4.50) | 4.53 | 0.11 | 0.03 | 0.03 | 0.91 |
| Period (0.20) | 0.15 | 0.08 | 0.05 | 0.01 | 0.92 |
| Trt1 (1.06) | 1.01 | 0.08 | 0.05 | 0.01 | 0.94 |
| Res1 (0.46) | 0.44 | 0.11 | 0.02 | 0.03 | 0.94 |
| Res2 (1.09) | 1.07 | 0.11 | 0.02 | 0.03 | 0.95 |
| Res3 (0.50) | 0.47 | 0.11 | 0.03 | 0.03 | 0.96 |
| $\sigma^2_e$ (1.44) | 1.42 | 0.07 | 0.02 | 0.01 | 0.92 |
| $\sigma^2_s$ (0.49) | 0.52 | 0.08 | 0.03 | 0.02 | 0.93 |

(iii) To test for the treatment effect we used, $H_0 : \beta_r = 0$ vs $H_1 : \beta_r \neq 0$

For empirical power computations we list the steps:

(a) For data simulation we used the true values of the parameters from Table 1. The proposed MCEM algorithm was used to find the estimates of the unknown parameters and variance components, $\theta$, and denoted as $\hat{\theta}_{full}$.

(b) To obtain the restricted estimates we maximised the Q-function under $H_0$, and computed $\hat{\theta}_{reduced}$.

(c) Using $\hat{\theta}_{full}$ and $\hat{\theta}_{reduced}$, the test statistic $T = 2Q(\hat{\theta}) - 2Q(\hat{\theta}_0)$ was determined and compared with $C = \chi^2_{(1-\alpha, df)}$, where df is the difference between the number of parameters in full and reduced model.

(d) The steps (a) – (d) were repeated 1000 times.

(e) The empirical power was computed as the proportion of times $H_0$ is rejected.

Figures 4 and 5 show the variation in the power function with respect to sample size and % of missingness (15%, 25% and 35%). From these plots
we note that power increases with sample size (or number of total subjects) and decreases with % of missingness. Note, power values in Figure 4 plot C are low due to the small effect size (difference between $\beta_{r_1}$ and $\beta_{r_3}$ is small). Similarly, in Figure 5 we see the power values decrease as we decrease the effect size (or as alternative value of $\beta_\tau$ decreases).

Figure 4: Power values of MCEM LRTs; The points plotted indicate the empirical proportion of test (by use of a nominal level $\alpha = 0.05$) that rejected the $H_0$ among 1000 simulated data sets. The plot on the left (A) correspond to the case with testing hypothesis on significance of response variates. Plots on the right (B, C, D) correspond to the case with testing hypothesis on pairwise comparison of response variates.
Figure 5: Power values of MCEM LRTs; The points plotted indicate the empirical proportion of test (by use of a nominal level $\alpha = 0.05$) that rejected the $H_0$ among 1000 simulated data sets. Plots A, B, C, D correspond to the case with testing hypothesis on treatment when alternative value of treatment effect is 1.06, -0.7, 0.5, 0.2, respectively.
7 Gene case study results

Based on the exploratory analysis discussed in Section 2, we fitted the following normal cross over model:

\[
y_{ijkl} = \beta_0 + \beta_1 \text{Period}_2 + \beta_2 \text{Period}_3 + \beta_3 \text{Trt}_2 + \beta_4 \text{Trt}_3 + \beta_5 \text{Gene}_2 \\
+ \beta_6 \text{Gene}_3 + \beta_7 \text{Gene}_4 + \beta_8 \text{Gene}_5 + \beta_9 \text{Gene}_6 + \beta_{10} \text{Gene}_7 \\
+ \beta_{11} \text{Gene}_8 + \beta_{12} \text{Gene}_9 + \beta_{13} \text{Gene}_10 + s_{ik} + e_{ijkl} \tag{7}
\]

where for the real data \(i, j = 1, 2, 3; l = 1(1)10\) and \(k = 1(1)n_i\), where \(n_1 = n_2 = 6\) and \(n_3 = 5\). The random effects \(s_{ik}\) and \(e_{ijkl}\) were assumed to be independent normal variates with variances \(\sigma_s^2\) and \(\sigma_e^2\), respectively. The period, treatment and gene effects were taken to be fixed and were represented by corresponding indicator variables. We assumed that the missing data mechanism is MAR.

Table 2 reports the maximum likelihood estimates of the parameters \((\beta, \sigma_s^2, \sigma_e^2)\) from the normal crossover model presented in eq. (7). For the MCEM algorithm, ANOVA estimates were used as initial values of the parameters. Standard errors of the estimates and their corresponding p-values obtained by the MCEM-LRT test procedure are also presented in section 4. One hundred imputations, each with burn-in of one thousand, were used for standard error estimation. In order to check the model adequacy computed the root mean square error (RMSE) of marginal residuals along with AIC and BIC values. The missing data estimates were compared with complete case analysis results.

From table 2, we observe that gene2, gene3, gene4, gene5, gene6, gene7, gene8, gene9, gene10 were significant at level of significance 0.10 in both complete case and missing case results. From the negative coefficients of the gene effects, we saw that the presence of that particular gene has a decreasing effect on the allergic reaction. Also, we note the AIC and BIC values of MAR responses model were much smaller as compared to complete case analysis. However, if instead of the responses we worked with log(responses) then the AIC, BIC values reduced further. From the RMSE of the residuals, we saw that the best fit was obtained by fitting the normal crossover model (eq. (7)) to log(responses).

We also artificially increased the number of missing responses from 9% to 21% and 24%. The corresponding results are given in table 3. We used a Bernoulli random variable for creating the artificially missing responses. For
the 21% case, we assumed that if there is a missing response for any gene in a specific period then all other responses are also missing. This mimicked the property of the actual real data set. However, in the 24% missingness case we assumed that in a specific period only some gene responses (not necessarily all) were missing. Under the higher missing percentage also, the gene effects were seen to be highly significant. The AIC, BIC and RMSE values for responses and log(responses) were also computed.

Table 2: Results based on the Gene data

| Variable | MLE   | SE    | p-value | MLE   | SE    | p-value | MLE   | SE    | p-value |
|----------|-------|-------|---------|-------|-------|---------|-------|-------|---------|
| Intercept| 3.49  | 0.02  | <0.0001 | 3.49  | 0.02  | <0.0001 | 1.25  | 0.01  | <0.0001 |
| Period$_2$| 0.01  | 0.01  | 0.96    | -0.02 | 0.01  | 0.73    | -0.003| 0.01  | 0.58    |
| Period$_3$| 0.01  | 0.01  | 0.70    | -0.01 | 0.01  | 0.58    | -0.003| 0.01  | 0.57    |
| Trt$_2$   | 0.01  | 0.01  | 0.57    | -0.01 | 0.01  | 0.58    | 0.002 | 0.01  | 0.61    |
| Gene$_2$  | -0.93 | 0.03  | <0.0001 | -0.94 | 0.02  | <0.0001 | -0.32 | 0.01  | <0.0001 |
| Gene$_3$  | -1.59 | 0.03  | <0.0001 | -1.59 | 0.02  | <0.0001 | -0.61 | 0.01  | <0.0001 |
| Gene$_4$  | -0.33 | 0.03  | <0.0001 | -0.33 | 0.02  | <0.0001 | -0.10 | 0.01  | <0.0001 |
| Gene$_5$  | -0.04 | 0.03  | 0.14    | -0.04 | 0.02  | 0.08    | -0.02 | 0.01  | 0.28    |
| Gene$_6$  | -1.27 | 0.03  | <0.0001 | -1.27 | 0.02  | <0.0001 | -0.45 | 0.01  | <0.0001 |
| Gene$_7$  | -1.48 | 0.03  | <0.0001 | -1.47 | 0.02  | <0.0001 | -0.55 | 0.01  | <0.0001 |
| Gene$_8$  | -2.03 | 0.03  | <0.0001 | -2.02 | 0.02  | <0.0001 | -0.87 | 0.01  | <0.0001 |
| Gene$_9$  | -0.82 | 0.03  | <0.0001 | -0.82 | 0.02  | <0.0001 | -0.27 | 0.01  | <0.0001 |
| Gene$_{10}$| -0.65 | 0.03  | <0.0001 | -0.63 | 0.02  | <0.0001 | -0.20 | 0.01  | <0.0001 |
| $s^2$      | 0.01  | 0.001 | -       | 0.01  | 0.001 | -       | 0.002 | 0.0001| -       |
| $s^2_\epsilon$| 0.001| 0.0004| -       | 0.001| 0.0004| -       | 0.0001| 0.0001| -       |

AIC -545.5  -1853.84 -2686.15
BIC -483.3  -1786.10 -2618.40
RMSE 0.11 0.11 0.05

8 Concluding remarks

In this paper we studied statistical model to analyse incomplete data in multivariate crossover setup. We presented the MCEM-LRT to test the fixed effects in the proposed set up, and exhibited the power analysis. This idea can be extended to non-ignorable missing (NMAR) case, where the parametric model for missing or the the missing data mechanism needs to be incorporated into the complete data log likelihood. As a consequence the estimation problem becomes more complex.

Acknowledgement We thank Dr. Atanu Bhattacharjee, Tata Memorial Center, Mumbai, India for his assistance in obtaining the gene data set.

Conflict of Interest The authors have declared no conflict of interest.
Table 3: Results based on the Gene data when we increase missing values

| Variable | MLE | SE  | p-value   | MLE | SE  | p-value   |
|----------|-----|-----|-----------|-----|-----|-----------|
|          |     |     | 21% missing |     |     | 24% missing |
| Intercept| 3.49| 0.02| < 0.0001  | 1.25| 0.01| < 0.0001  |
| Period_2 | -0.003| 0.01| 0.48   | -0.003| 0.004| 0.39   |
| Period_3 | 0.01| 0.01| 0.28   | 0.005| 0.004| 0.21   |
| Treatment_2 | -0.001| 0.01| 0.53   | -0.0004| 0.004| 0.57   |
| Treatment_3 | 0.003| 0.01| 0.74   | 0.001| 0.004| 0.53   |
| Gene_2   | -0.93| 0.02| < 0.0001| -0.31| 0.01| < 0.0001 |
| Gene_3   | -1.60| 0.02| < 0.0001| -0.61| 0.01| < 0.0001 |
| Gene_4   | -0.33| 0.02| < 0.0001| -0.09| 0.01| < 0.0001 |
| Gene_5   | -0.04| 0.02| 0.06   | -0.01| 0.01| 0.19   |
| Gene_6   | -1.27| 0.02| < 0.0001| -0.45| 0.01| < 0.0001 |
| Gene_7   | -1.47| 0.02| < 0.0001| -0.55| 0.01| < 0.0001 |
| Gene_8   | -2.02| 0.02| < 0.0001| -0.86| 0.01| < 0.0001 |
| Gene_9   | -0.82| 0.02| < 0.0001| -0.27| 0.01| < 0.0001 |
| Gene_10  | -0.63| 0.02| < 0.0001| -0.20| 0.01| < 0.0001 |
| σ²_e     | 0.01| 0.001| -      | 0.002| 0.001| -      |
| σ²_s     | 0.001| 0.0003| -     | 0.0001| 0.0001| -     |
| AIC       | -1907.3| -2758.3|        |   |
| BIC       | -1839.6| -2690.5|        |   |
| RMSE      | 0.09| 0.04|          |         | |
References

[1] Sanjib Basu and Sourav Santra. A joint model for incomplete data in crossover trials. *Journal of Statistical Planning and Inference*, 140(10): 2839–2845, 2010.

[2] Vernon M. Chinchilli and James D. Esinhart. Design and analysis of intra-subject variability in cross-over experiments. *Statistics in Medicine*, 15:1619–1634, 1996.

[3] Shein-chung Chow and Jun Shao. Statistical methods for two-sequence three-period cross-over designs with incomplete data. *Statistics in Medicine*, 16:1031–1039, 1997.

[4] Emily Clough and Tanya Barrett. The Gene Expression Omnibus database. *Methods in Molecular Biology*, 1418:93–110, 2016.

[5] Goetghebeur Els and Louise Ryan. Semiparametric regression analysis of interval-censored data. *Biometrics*, 56(4):1139–1144, 2000.

[6] Julie M. Grender and William D. Johnson. Analysis of crossover designs with multivariate response. *Statistics in Medicine*, 12(1):69–89, 1993.

[7] Weang K. Ho, John N.S. Matthews, Robin Henderson, Daniel Farewell, and Lauren R. Rodgers. Dropouts in the AB/BA crossover design. *Statistics in Medicine*, 31(16):1675–1687, 2012.

[8] Joseph G. Ibrahim and Geert Molenberghs. Missing data methods in longitudinal studies: A review. *Test*, 18(1):1–43, 2009.

[9] Byron Jones and Michael G. Kenward. *Design and Analysis of Cross-Over Trials*. Chapman & Hall/CRC, second edition, 2003.

[10] Nan M. Laird. Missing data in longitudinal studies. *Statistics in Medicine*, 7:305–315, 1988.

[11] BR Leaker, VA Malkov, R Mogg, MK Ruddy, GC Nicholson, AJ Tan, C Tribouley, and G Chen. The nasal mucosal late allergic reaction to grass pollen involves type 2 inflammation (IL-5 and IL-13), the inflammasome (IL-1β), and complement. *Nature*, 10(2):408–420, 2016.

[12] John N.S. Matthews and Robin Henderson. Two-period, two-treatment
crossover designs subject to non-ignorable missing data. *Biostatistics*, 14(4):626–638, 2013.

[13] H. I. Patel. Analysis of incomplete data in a two-period crossover design with reference to clinical trials. *Biometrika*, 72(2):411–418, 1985.

[14] Mary Putt and Vernon M. Chinchilli. A mixed effects model for the analysis of repeated measures cross-over studies. *Statistics in Medicine*, 18(22):3037–3058, 1999.

[15] Barbra A. Richardson and Virginia F. Flack. The analysis of incomplete data in the three-period two-treatment crossover design for clinical trials. *Controlled Clinical Trials*, 13(5):381, 1992.

[16] Gerd K. Rosenkranz. Analysis of cross-over studies with missing data. *Statistical Methods in Medical Research*, 24(4):420–433, 2015.

[17] Stephen Senn. *Cross-Over Trials in Clinical Research*. John Wiley & Sons, Ltd., 2002.

[18] Jun Shao. *Mathematical Statistics*. Springer International Publishing, second edition, 2003.

[19] Gail E. Tudor, Gary G. Koch, and Diane Catellier. 20 Statistical methods for crossover designs in bioenvironmental and public health studies. *Handbook of Statistics*, 18(1980):571–614, 2000.

[20] Greg C.G. Wei and Martin A. Tanner. A Monte Carlo Implementation of the EM Algorithm and the Poor Man’s Data Augmentation Algorithm. *Journal of the American Statistical Association*, 85(411):699–704, 1990.